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Ralph-Sydney Mboumba Bouassa

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Université de Paris

Ecole doctorale BioSPC (ED : 562)

Epidémiologie et immunité muqueuse spécifique au cours de

l'infection à Papillomavirus Humains:

Implications pour la prévention des cancers associés

Par Ralph-Sydney MBOUMBA BOUASSA

Thèse de doctorat en Immunologie

Spécialité : Immunologie

Dirigée par Mr Laurent BELEC

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Devant un jury composé de :

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Mr Jérôme Le GOFF, PU-PH, Université Paris Diderot	Examineur
Mr Laurent BELEC, PU-PH, Université Paris Descartes	Directeur

Titre : Epidémiologie et immunité muqueuse spécifique au cours de l'infection à Papillomavirus Humains: Implications pour la prévention des cancers associés

Résumé : Les papillomavirus humain (HPV) à haut-risque (HR-HPV) oncogène [groupes $\alpha 7$ (HPV-18, -45 et -68) et $\alpha 9$ (HPV-16, -31, -33, -35, -52 et -58)] sont les agents étiologiques du cancer du col de l'utérus, qui est désormais le premier cancer féminin dans plusieurs pays d'Afrique subsaharienne. Notre premier objectif a été de construire un corpus d'hypothèses de travail de recherche issus des données de la littérature, en rédigeant deux revues extensives de la littérature, l'une sur le cancer du col en Afrique, et l'autre sur l'immunité humorale systémique et muqueuse spécifique contre les HPV. Notre deuxième objectif a été de recueillir des collections biologiques à partir de cohortes cliniques d'individus Africains à risque d'infection à HPV oncogènes, en Afrique subsaharienne (République Centrafricaine; Tchad) et en France (femmes immigrées de 1ère génération). Notre troisième objectif a été d'acquérir et de transférer des techniques sophistiquées de virologie médicale (détection et génotypage des HPV par PCR en temps réel multiplex) et d'immunologie [production de "virus-like particles" (VLP) et mise au point d'un test sérologique de détection des immunoglobulines systémiques et muqueuses contre les HR-HPV des groupes $\alpha 7/\alpha 9$]. Notre quatrième objectif a été d'appliquer les techniques moléculaires acquises afin de décrire l'épidémiologie moléculaire de l'infection à HPV de cohortes d'individus Africains inclus (femmes du Tchad; Africaines immigrées vivant en France; homosexuels masculins de Centrafrique) et de prédire au sein de celles-ci l'efficacité de la vaccination prophylactique multivalente contre les HPV. Nous avons ainsi démontré au sein de ces cohortes : i) des prévalences particulièrement élevées d'infection(s) à HPV oncogènes et d'infection à VIH-1; ii) une épidémiologie moléculaire des infections à HPV oncogènes inédite, avec une distribution des HR-HPV très différente de celle connue dans les pays développés; et enfin iii) une efficacité prédictive potentielle de la vaccination prophylactique par le vaccin nonavalent Gardasil-9®. Enfin, notre cinquième et dernier objectif a été d'appliquer les techniques immunologiques acquises ("VLP-based ELISA") pour évaluer la réponse immunitaire à IgG systémique et muqueuse contre les HR-HPV des groupes $\alpha 7/\alpha 9$, dans le but de poser les bases immunologiques de la possibilité de vaccination de rattrapage par les vaccins prophylactiques contre les HPV oncogènes au sein des femmes Africaines immigrées de 1ère génération vivant en France, qui sont à risque particulièrement élevé de cancer du col de l'utérus. Nous avons ainsi démontré l'existence de 3 catégories de femmes: a) une première catégorie majoritaire ($\approx 2/3^{\circ}$ des femmes), montrant une répllication génitale à HPV associée à une réponse immunitaire à IgG systémique et génitale contre la plupart des $\alpha 7/\alpha 9$ HR-HPV ciblés par le vaccin Gardasil-9®, avec des réactivités croisées contre la plupart des antigènes

des $\alpha 7/\alpha 9$ HPV d'intérêt, témoignant probablement d'antécédents de multiples épisodes infectieux génitaux à HPV en plus de l'infection génitale actuelle; cette catégorie de femmes ne pourrait pas a priori bénéficier de la vaccination prophylactique multivalente de rattrapage contre les HPV oncogènes ; b) une deuxième catégorie ($\approx 1/5^\circ$) montrant l'absence d'infection génitale à HPV associée à une faible réponse immunitaire à IgG systémique et génitale dirigée uniquement contre les $\alpha 9$ HPV ciblés par le Gardasil-9®; cette catégorie de femmes pourrait éventuellement bénéficier de la vaccination de rattrapage contre les HPV; et enfin c) une troisième catégorie ($\approx 1/10^\circ$) montrant l'absence conjointe d'infection génitale à HPV et de réponse immunitaire à IgG systémique et génitale contre les $\alpha 7/\alpha 9$ HPV ciblés par le Gardasil-9®; malgré leur âge avancé, cette catégorie de femmes pourrait bénéficier pleinement de la vaccination prophylactique contre les HPV.

Mots clefs : Cancer du col de l'utérus ; Papillomavirus humains oncogènes ; $\alpha 7/\alpha 9$ HR-HPV ; Afrique sub-Saharienne ; Populations africaines à risque ; Epidémiologie moléculaire ; Femmes immigrées africaines vivant en Europe ; Réponse immunitaire humorale ; IgG systémique et génitale ; Virus-like particles (« VLP ») ; Gardasil-9® ; Vaccination de rattrapage ; Vaccination HPV prophylactique.

Title : Epidemiology and Specific Mucosal Immunity During Human Papillomavirus Infection: Implications for the Prevention of Associated Cancers

Abstract : High-risk Human papillomavirus (HR-HPV) [groups $\alpha 7$ (HPV-18, -45 and -68) and $\alpha 9$ (HPV-16, -31, -33, -35, -52 and -58)] are the causative agents of cervical cancer, which is now the first female cancer in several countries in sub-Saharan Africa. Our first objective was to build a body of research hypotheses based on literature data, by writing two extensive reviews of the literature, one on cervical cancer in Africa, and the other on systemic and mucosal humoral immunity against HPV. Our second objective was to collect biological collections from clinical cohorts of Africans at risk for oncogenic HPV infection, both in sub-Saharan Africa (Central African Republic and Chad) and in France (1st generation African immigrant women). Our third goal consisted in the acquisition and transfer of sophisticated techniques of medical virology (detection and genotyping of HPV by multiplex real-time PCR) and immunology [production of "virus-like particles" (VLPs) and development of a serological test for the detection of systemic and mucosal immunoglobulins against HR-HPV groups $\alpha 7/\alpha 9$]. Our fourth objective was to apply the acquired molecular techniques to describe the molecular epidemiology of HPV infection in cohorts of African individuals (Chadian women, African immigrants living in France, Central African homosexual males) and to predict within these groups, the efficacy of multivalent prophylactic vaccination against HPV. In these cohorts we demonstrated: i) particularly high prevalence of oncogenic HPV infection(s) and HIV-1 infection; (ii) atypical molecular epidemiology of oncogenic HPV infections, with an HR-HPV distribution very different from that commonly reported in developed countries; and lastly, iii) a potential predictive efficacy of prophylactic vaccination with Gardasil-9[®] vaccine. Finally, our fifth and last objective was to apply the acquired immunological techniques ("VLP-based ELISA") to evaluate the systemic and mucosal IgG responses against the HR-HPV groups $\alpha 7/\alpha 9$, in order to propose immunological basis for the possibility of catch-up vaccination with prophylactic vaccines against oncogenic HPV among first-generation African women living in France, who are at particularly high-risk of cervical cancer. We thus demonstrated the existence of 3 categories of women: a) a first one, which was the predominant category ($\approx 2/3^{\circ}$ of women), showing genital HPV replication associated with a systemic and genital IgG immune response against most $\alpha 7/\alpha 9$ HR-HPV targeted by the Gardasil-9[®] vaccine, with cross-reactivities against most of the $\alpha 7/\alpha 9$ HPV antigens of interest, probably reflecting a history of multiple episodes of HPV genital infection in addition to present genital infection; this category of women could not a priori benefit from the multivalent prophylactic catch-up vaccination against oncogenic HPV; b) a second

category ($\approx 1/5^\circ$) showing the absence of genital HPV infection associated with a weak systemic and genital IgG immune response against only the $\alpha 9$ HPV targeted by the Gardasil-9®; this category of women could possibly benefit from the catch-up vaccination against HPV; and finally (c) a third category ($\approx 1/10^\circ$) showing the absence of genital HPV infection and of the systemic and genital IgG immune response against $\alpha/\alpha 9$ HPV targeted by the Gardasil-9®; despite their advanced age, the Gardasil-9® would be fully efficient in protecting these women. Thus, this category of women could fully benefit from prophylactic vaccination against HPV.

Keywords : Cervical cancer; Oncogenic human papillomaviruses; $\alpha 7/\alpha 9$ HR-HPV; Sub-Saharan Africa ; At-risk African populations; Molecular epidemiology; African immigrant women living in Europe; Humoral immune response; Systemic and genital IgG; Virus-like particles ("VLPs"); Gardasil-9®; Catch-up vaccination; Prophylactic HPV vaccination.

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*« Ô Dieu, tu voulais savoir ce que nous valons.
Aussi comme on purifie l'argent au feu,
tu nous as purifié par la souffrance »*

Parole de Vie, Psaumes 66.10.

Sommaire

Liste des tableaux et figures

Partie 1 : Synthèse bibliographique	1
I. Biologie des Papillomavirus humains	2
1. Biologie des Papillomavirus.....	2
2. Structure de la particule virale.....	2
3. Organisation du génome viral et fonction des protéines virales.....	3
a. Organisation du génome viral.....	3
b. Fonction des protéines virales.....	4
4. Classification.....	5
II. Epidémiologie de l'infection génitale à HPV chez la femme en Afrique subsaharienne	8
III. Immunité humorale naturelle et vaccino-induite dans les compartiments systémiques et muqueux au cours de l'infection à Papillomavirus humains	24
IV. Problématique et objectifs de l'étude	81
Partie 2 : Mémoire expérimental	83
I. Matériel et Méthodes	84
1. Conception de l'étude.....	84
2. Population de l'étude.....	84
a. Protocole GYNAUTO TCHAD.....	84
b. Protocole IMMIPAP ANRS.....	89
c. Protocole HOMOPAP RCA.....	91
3. Analyses de biologie moléculaire et sérologiques.....	93
a. Analyses de biologie moléculaire.....	93
i. Extraction des acides nucléiques, détection et génotypages des HPV.....	93
b. Analyses sérologiques.....	93
i. Production des antigènes viraux pour les analyses sérologiques : [HPV major capsid antigen L1 Virus-like particles (VLP)].....	93
ii. Détermination de la séropositivité vis-à-vis de l'infection à HPV par Enzyme linked immunosorbent assay (ELISA).....	95

II. Résultats.....	97
A. Epidémiologie moléculaire de l'infection à HPV au sein de populations africaines à risque.....	97
1. Forte prévalence de l'infection cervicale à Papillomavirus humains à haut-risque, en particulier par ceux couverts par le vaccin prophylactique Gardasil-9 [®] , au sein d'une population de femmes adultes vivant à N'Djamena, au Tchad.....	97
2. Forte prévalence de l'infection cervicale à Papillomavirus humains à haut-risque, causée par des génotypes atypiques, au sein d'une population de femmes adultes, immigrées de première génération, originaires d'Afrique sub-saharienne et vivant en France.....	120
3. Distribution anale inhabituelle et unique de génotypes à haut-risque de Papillomavirus humains (HR-HPV) parmi des hommes ayant des rapports sexuels avec des hommes vivant en République Centrafricaine.....	140
4. Efficacité d'un dispositif d'auto-prélèvement (voile vaginal) pour le dépistage des infections à HPV par PCR multiplex en temps réel chez des femmes adultes vivant en Afrique subsaharienne.....	160
B. Caractérisation de la réponse immunitaire humorale induite à la suite d'une infection à Papillomavirus humains.....	200
1. Caractérisation de la réponse immunitaire IgG génitale et sérique contre les génotypes de HPV appartenant aux groupes $\alpha 7$ et $\alpha 9$ chez des femmes adultes non-vaccinées non-éligibles pour la vaccination HPV selon les recommandations vaccinales actuelles.....	200
Discussion, conclusions et perspectives.....	251
A. Prévalence et distribution des principaux génotypes HR-HPV au sein de populations africaines à risque pour l'infection à HPV.....	252
B. Infection génitale à HPV et réponse immunitaire humorale acquise chez des femmes immigrées africaines vivant en France.....	259
C. Implications pour la révention des cancers HPV-induits: Adaptation des recommandations internationales de prévention contre les infections à HPV et les cancers associés pour les populations à très haut risque	263
1. Profils immunovirologiques et éligibilité pour le rattrapage de la vaccination contre l'infection à HPV.....	263
2. Auto-prélèvement des sécrétions génitales pour le diagnostic moléculaire de l'infection à HPV.....	266

Bibliographie.....269

Annexes.....344

Liste des figures et tableaux

I. Liste des figures

• **Biologie des Papillomavirus humains**

- Figure 1. Structure des papillomavirus.....2
- Figure 2. Représentation schématique du génome circulaire du HPV-16.....3
- Figure 3. Classification phylogénétique des papillomavirus.....6
- Figure 4. Arbre phylogénétique HPV16 basé sur 99 séquences E6/LCR.....7

• **Epidémiologie de l'infection génitale à HPV chez la femmes en Afrique subsaharienne**

- Figure 1. Course of HPV infections, cervical lesions and prevention strategies.....10
- Figure 2. Burden of cervical cancer in Africa.....10
- Figure 3. Evolution of forecasting of maternal and cervical cancer-related mortalities between 1990 and 2030 in the world.....11
- Figure 4. HPV vaccination in cervical cancer prevention.....16
- Figure 5. African progress in HPV vaccination.....16
- Figure 6. strategies and intervention for cervical cancer prevention in Africa.....18

• **Immunité humorale naturelle et vaccino-induite dans les compartiments systémiques et muqueux au cours de l'infection à Papillomavirus humains**

- Figure 1. Natural history of HPV infection and viral cycle.....30
- Figure 2. Humoral response mechanism induced by HPV vaccination.....34
- Figure 3. Schematic representation of the complex immune response to Gardasil® HPV prophylactic vaccine.....39
- Figure 4. From native human papillomavirus (HPV) to virus-like particles (VLPs)-based HPV prophylactic vaccines.....66
- Figure 5. Demonstration of HPV-16/-18 neutralizing antibody responses in vaccinated women.....69

• **Materiel et méthodes**

- Figure 5. Diagramme directionnel de la réalisation du protocole de recherche GYNAUTO.....88
- Figure 6. Diagramme directionnel de la réalisation du protocole de recherche IMMIPAP.....91
- Figure 7. Diagramme directionnel de la réalisation du protocole de recherche HOMOPAP.....92

• **Forte prévalence de l'infection cervicale à Papillomavirus humains à haut-risque, en particulier par ceux couvert par le vaccin prophylactique Gardasil-9, au sein d'une population de femmes adultes vivant à N'Djamena, au Tchad.**

Figure 1. Map showing the location of 23 inclusion study sites in N'Djamena, Chad.....	102
Figure 2. Distribution of HPV genotypes according to their inclusion in the 9-valent Gardasil-9 vaccine.....	107
Figure 3. Distribution of HPV types in 57 adult women.....	108
• Forte prévalence de l'infection cervicale à Papillomavirus humains à haut-risque, causée par des génotypes atypiques, au sein d'une population de femmes adultes, immigrées de première génération, originaires d'Afrique sub-saharienne et vivant en France.	
Figure 1. Proportion of cervical HPV genotypes according to their inclusion in the 9-valent Gardasil-9® vaccine.....	130
• Distribution anale inhabituelle et unique de génotypes à haut-risque de Papillomavirus humains (HR-HPV) parmi des hommes ayant des rapports sexuels avec des hommes vivant en République Centrafricaine.	
Figure 1. Distribution of anal HPV types included (or not included) in the 9-valent Gardasil-9® vaccine.....	148
Figure 2. Distribution of anal HPV types according to the HIV-serostatus.....	149
• Efficacité d'un dispositif d'auto-prélèvement (voile vaginal) pour le dépistage des infections à HPV par PCR multiplex en temps réel chez des femmes adultes vivant en Afrique subsaharienne	
Figure 1. Flow diagram of the GYNAUTO-CHAD study.....	167
Figure 2. V-Veil-Up Gyn Collection Device (V-Veil-Up Pharma Ltd.).....	169
Figure 3. Flow diagram of study recruitment, specimen collection, and HPV test results by multiplex real-time PCR.....	174
Figure 4. Percentages of detection by multiplex real-time PCR assay Anyplex™ II HPV28.....	179
Figure 5. Distribution of the 28 HPV genotypes detected by multiplex real-time PCR assay Anyplex™ II HPV28.....	180
Figure 6. Relevant examples of paired results obtained by gold standard clinician-collected endocervical swab and self-collected veil.....	182
• Caractérisation de la réponse immunitaire IgG génitale et sérique contre les génotypes de HPV appartenant aux groupes $\alpha 7$ et $\alpha 9$ chez des femmes adultes non-vaccinées non-éligibles pour la vaccination HPV selon les recommandations vaccinales actuelles	
Figure 1. Flow diagram of the ImmiPap study.....	210

Figure 2. Prevalences of IgG reactivities to $\alpha 7/\alpha 9$ HPV types in paired serum and cervicovaginal secretions from study women with positive HPV DNA detection in their genital tract (group I; N=35) (black hatched boxes), and from women with negative cervicovaginal HPV DNA detection (group II; N=16) (white boxes).	220
Figure 3. Prevalences of IgG reactivities to $\alpha 7/\alpha 9$ HPV types in paired serum (black boxes) and cervicovaginal secretions (grey boxes) according the presence of genital HPV DNA.....	221

II. Liste des tableaux

- **Biologie des Papillomavirus humains**

Tableau 1. Comparaison de la nomenclature des papillomavirus selon l’ICTV et celle communément utilisée.....	11
--	----

- **Epidémiologie de l’infection génitale à HPV chez la femmes en Afrique subsaharienne**

Table 1. Primary, secondary and tertiary preventive strategies according to age groups.....	13
---	----

Table 2. Screening tests for secondary prevention of human papillomavirus (HPV) genital infections in women.....	13
--	----

Table 3. African countries where Gardasil® is approved (including GAVI-eligible African countries).....	17
---	----

- **Immunité humorale naturelle et vaccino-induite dans les compartiments systémiques et muqueux au cours de l’infection à Papillomavirus humains**

Table 1. Advantages and constraints of HPV serological assays.....	40
--	----

Table 2 (Annexe 1). Principal studies reporting humoral immune response to HPV in serum, genital and saliva samples during natural HPV infection.....	345
---	-----

- **Forte prévalence de l’infection cervicale à Papillomavirus humains à haut-risque, en particulier par ceux couvert par le vaccin prophylactique Gardasil-9, au sein d’une population de femmes adultes vivant à N’Djamena, au Tchad.**

Table 1. Basline characteristics of 253 adult women living in N’Djamena, in Chad.....	105
---	-----

Table 2. Logistic regression analyses for cervical HPV-associated risk factors in 253 adult women living in N’Djamena, in Chad.....	109
---	-----

- **Forte prévalence de l’infection cervicale à Papillomavirus humains à haut-risque, causée par des génotypes atypiques, au sein d’une population de femmes adultes,**

immigrées de première génération, originaires d’Afrique sub-saharienne et vivant en France.

Table 1. Baseline characteristics of 50 African immigrant women living in Orléans, France.....127

Table 2. Characteristics of the study women according to their HIV serostatus.....129

• **Distribution anale inhabituelle et unique de génotypes à haut-risque de Papillomavirus humains (HR-HPV) parmi des hommes ayant des rapports sexuels avec des hommes vivant en République Centrafricaine.**

Table 1. Baseline characteristics according to sexual behavior and HIV serostatus among the 42 study men who have sex with men (MSM) living in Bangui, Central African Republic.....147

Table 2. Univariate and multivariate logistic regression analyses for HPV-associated risk factors in 42 study men who have sex with men (MSM) living in Bangui, Central African Republic.....150

• **Efficacité d'un dispositif d'auto-prélèvement (voile vaginal) pour le dépistage des infections à HPV par PCR multiplex en temps réel chez des femmes adultes vivant en Afrique subsaharienne**

Table 1. (Annexe 2) Baseline characteristics of the 253 study adult women living in N'Djamena, in Chad.....349

Table 2. Acceptability of veil-based self-sampling using the V-Veil-Up Gyn Collection Device (V-Veil-Up Pharma Ltd.) compared to swab-based clinician-collection for HPV DNA testing among 253 study women living in N'Djamena, Chad.....175

Table 3. Satisfaction questionnaire regarding veil-based self-sampling using the V-Veil-Up Gyn Collection Device (V-Veil-Up Pharma Ltd.) among 253 study women living in N'Djamena, Chad.....176

Table 4. Two-by-two tables of cervicovaginal specimens self-collected using the V-Veil-Up Gyn Collection Device (V-Veil-Up Pharma Ltd.) compared to clinician-collected endocervical swab specimens for the detection by multiplex real-time PCR of any HPV genotypes, HR-HPV genotypes and HPV genotypes targeted by the 9-valent Gardasil-9® vaccine (Merck & Co. Inc.).....177

Table 5 (Annexe 3). Correspondence between the 7,084 (= 253 x 28) results obtained from the 253 paired swab-collected and veil-collected genital specimens to detect the 28 HPV genotypes included in Anyplex™ II HPV28 kit (Seegene).....351

Table 6. Correspondence between the 3,289 (= 253 x 13) results obtained from the 253 paired swab-collected and veil-collected genital specimens to detect the 13 HR-HPV genotypes included in Anyplex™ II HPV28 kit (Seegene).....181

- **Caractérisation de la réponse immunitaire IgG génitale et sérique contre les génotypes de HPV appartenant aux groupes $\alpha 7$ et $\alpha 9$ chez des femmes adultes non-vaccinées non-éligibles pour la vaccination HPV selon les recommandations vaccinales actuelles**

Table 1. Sociodemographic characteristics, HIV serostatus, past-history of sexually transmitted infections and cervical cytological results in study women, according the presence or absence of HPV DNA detected by multiplex real-time PCR in swab-collected cervicovaginal secretions.....215

Table 2. HPV-specific IgG immune response in systemic and cervicovaginal compartments, correspondence between HPV types in genital secretions and serum or cervicovaginal IgG reactivities and cross-reactivity of serum or cervicovaginal HPV-specific IgG against HPV types different from detected cervicovaginal DNA HPV, among study women with positive HPV DNA detection in their cervicovaginal secretions (group I; N=35), including women with HR-HPV DNA (group Ia; N=29) and women with LR-HPV DNA (group Ib; N=6), and among women with negative HPV DNA detection in their cervicovaginal secretions (group II; N=16).The grey cases in group Ia correspond to concordance between HR-HPV DNA types and serum or cervicovaginal HPV-specific IgG.....217

Table 3 (Annexe 4). Number of serum or cervicovaginal IgG seropositivities to $\alpha 7/\alpha 9$ HR-HPV types included in the nonavalent Gardasil-9® vaccine among study women with positive HPV DNA detection in their cervicovaginal secretions (group I; N=35), including women with HR-HPV DNA (group Ia; N=29) and women with LR-HPV DNA (group Ib; N=6), and among women with negative HPV DNA detection in their cervicovaginal secretions (group II; N=16).....352

Partie 1

Synthèse bibliographique

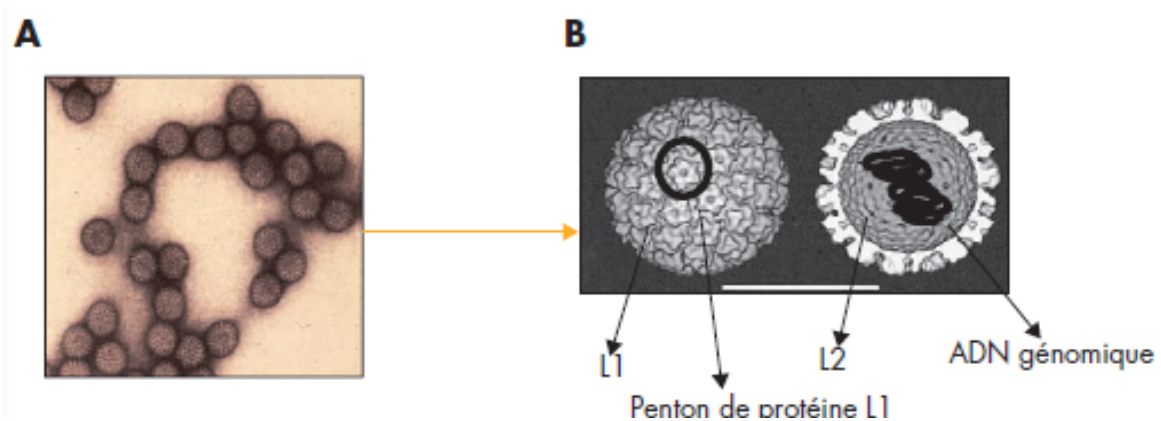
I. Biologie des Papillomavirus humains

1. Biologie des Papillomavirus humains

Les papillomavirus humains (HPV) sont des virus très communs. Il en existe plus de 200 types. Les papillomavirus sont des virus épithéliotropes qui infectent les cellules de la peau et des muqueuses et sont responsables de lésions bénignes telles que des verrues vulgaires et plantaires, des verrues génitales et des condylomes acuminés, mais également des lésions malignes telles que des cancers de la peau lié à l'épidermodysplasie verruciforme et des carcinomes anogénitaux, notamment le cancer du col de l'utérus, 1er cancer de la femme africaine (Mboumba Bouassa et al., 2017).

2. Structure de la particule virale

Les HPV sont un groupe hétérogène de virus non enveloppés, de taille comprise entre 52 et 55 nm (Van Doorslaer et al., 2018). La capside des HPV est composée de 72 structures protéiques élémentaires appelées capsomères disposés selon une symétrie icosaédrique. Chaque capsomère est constitué par l'auto-assemblage de la protéine majeure "Late protein 1" (L1) de 55 kDa, représentant approximativement 90% du poids total des protéines virales et d'une protéine mineure "Late protein 2" (L2) (Figure 1). Ces protéines forment la structure du virion et facilitent le conditionnement et la maturation de l'ADN viral (Doorbar & Griffin 2007; Senapati et al., 2016; Van Doorslaer et al., 2018).



3. Organisation du génome viral et fonction des protéines virales

a. Organisation du génome viral

Le génome des papillomavirus est constitué d'un ADN double brin circulaire d'environ 8000 paires de bases (bp), super-enroulé et associé à des histones cellulaires (Van Doorslaer et al., 2018). Il est organisé principalement en trois régions (Figure 2) :

- ✓ Une région régulatrice en amont ou "Long control region" (LCR), qui contient des séquences qui contrôlent la transcription et la réplication virales ;
- ✓ Une région précoce, qui contient les cadres de lecture ouverts (ORF) des gènes précoces (E1, E2, E4, E5, E6 et E7) impliqués dans de multiples fonctions, notamment la transactivation de la transcription, la transformation, la réplication et l'adaptation virale à différents milieux cellulaires ;
- ✓ Une région tardive, qui code pour les protéines de capside (L1 et L2) (Van Doorslaer et al., 2018).

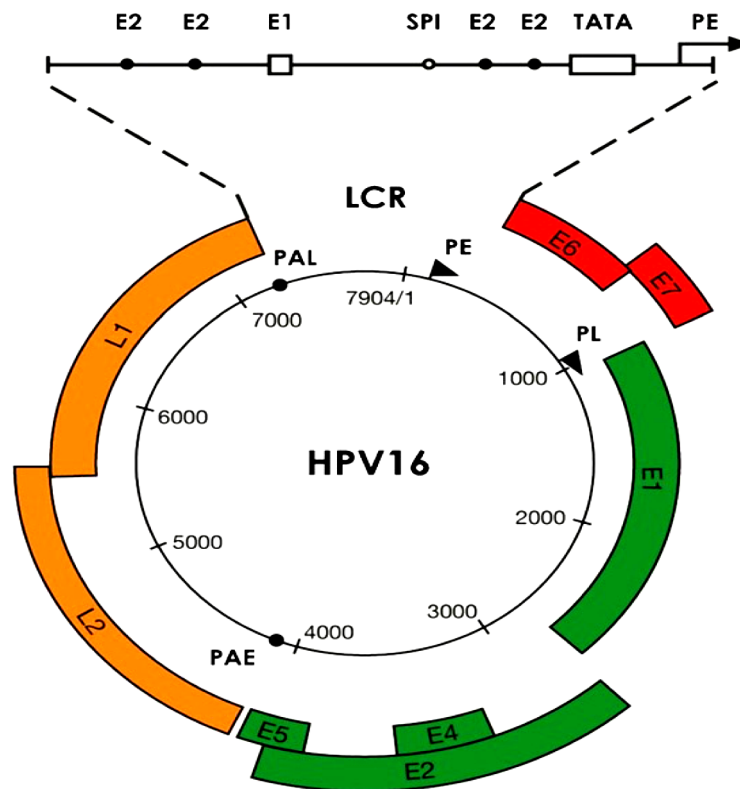


Figure 2. Représentation schématique du génome circulaire du HPV-16. Les deux promoteurs sont illustrés par des flèches noires. Les gènes précoces sont représentés en rouge ou vert (E1, E2, E4, E5, E6 et E7). Les gènes tardifs (L1 et L2) sont en orange et les sites de liaison des facteurs de transcription sont indiqués dans le LCR.

b. Fonction des protéines virales

➤ La protéine E1

Le gène qui code la protéine E1 (73 kDa) constitue une des portions du génome viral les plus conservées chez tous les HPV. Cette protéine E1 est impliquée dans le processus de réplication virale. Elle exerce la fonction de reconnaissance de l'origine de réplication et possède à la fois une activité ATPase et une fonction hélicase (Doorbar & Griffin 2007; Senapati et al., 2016).

➤ La protéine E2

La protéine E2 a une longueur d'environ 400 à 500 résidus d'acides aminés (AA). Elle joue plusieurs rôles dans le cycle viral. E2 est le principal régulateur transcriptionnel du virus et peut à la fois activer et réprimer la transcription virale en se liant spécifiquement à des motifs consensus dans le génome viral (ACNNNNNGGT ou ACCGNNNCGGT) appelé E2BS (E2 binding site) (Doorbar & Griffin 2007; Senapati et al., 2016). Elle initie également la réplication de l'ADN viral en chargeant la protéine E1 hélicase sur l'origine de réplication. L'intégration du génome du virus dans celui de l'hôte se fait également par le clivage de la protéine E2. Enfin, la protéine E2 régule la transcription des gènes E6 et E7 (Doorbar & Griffin 2007; Senapati et al., 2016).

➤ La protéine E4

La protéine E4 possède à la fois une forme majeure de 17 kDa, et deux formes mineures de 16 et 10 kDa. Cette protéine intervient dans la stabilisation du génome viral ainsi que dans la régulation de l'expression des gènes tardifs. Elle contribue également au contrôle de la maturation et de la libération des particules virales (Doorbar & Griffin 2007).

➤ La protéine E5

La protéine E5 possède un pouvoir transformant mais considéré plus faible chez les humains que celui exercé chez les bovins, elle participe aussi, avec les protéines E6 et E7, à l'inhibition de la réponse immunitaire de l'hôte à l'infection virale (Senapati et al., 2016).

➤ La protéine E6

La protéine E6 est composée d'environ 160 acides aminés et possède quatre motifs Cys-X-X-Cys permettant la fixation d'un atome de Zinc. La principale propriété de la protéine E6 est son interaction avec la protéine cellulaire suppresseur de tumeur p53. De plus, la protéine E6 a d'autres cibles cellulaires qui favorisent également l'immortalisation des cellules, par le

maintien de la longueur des télomères après activation de la télomérase (Doorbar & Griffin 2007; Senapati et al., 2016).

➤ **La protéine E7**

C'est une protéine composée de 100 acides aminés, retrouvée majoritairement dans le noyau et pouvant lier des atomes de zinc grâce à ses deux motifs Cys-X-X-Cys. Le rôle central de la protéine E7 est son habilité à se lier avec la protéine du rétinoblastome (pRB). Elle est impliquée dans le processus de régulation du cycle cellulaire. L'extrémité N-terminale de la protéine E7 possède deux séquences CR1 et CR2 qui servent de sites de fixation aux protéines pRB, p100 et p130 (Doorbar & Griffin 2007; Senapati et al., 2016).

4. Classification

Les papillomavirus appartiennent à la famille des *Papillomaviridae*. Cette famille comprend deux sous-familles, *Firstpapillomavirinae*, qui comprend plus de 50 genres et plus de 130 espèces, et *Secondpapillomavirinae*, avec un seul genre et une seule espèce (Van Doorslaer et al., 2018). Les genres sont nommés d'après l'alphabet grec : alpha, beta, gamma, mu et nu et les espèces sont identifiées par le nom du genre correspondant, associé à un nombre entier (alpha1, alpha2, etc.) (Figure 3) (de Villiers et al., 2004). La classification des HPV est basée sur la variabilité au sein du gène L1. Selon cette classification, on compte à ce jour plus de 200 types de HPV appartenant aux genres alpha, bêta, gamma, mu et nu (de Villiers et al., 2004). Ainsi, lorsque deux séquences L1 diffèrent l'une de l'autre de plus 90 %, deux génotypes différents sont ainsi définis. Une espèce regroupe les types présentant une homologie supérieure à 70 % dans leur gène L1, et l'appartenance à un même genre est définie par une homologie supérieure à 60 % (de Villiers et al., 2004; Bernard et al., 2006). En outre, pour qu'un nouveau type d'HPV soit reconnu, il faut que le génome complet du virus ait été séquencé et que sa séquence L1 présente une divergence de plus de 10 % avec la séquence L1 du type connu le plus proche génétiquement. Une différence de 2 à 10 % définit l'appartenance à un sous-type et une différence de moins de 2 % définit un variant (de Villiers et al., 2004; Bernard et al., 2006).

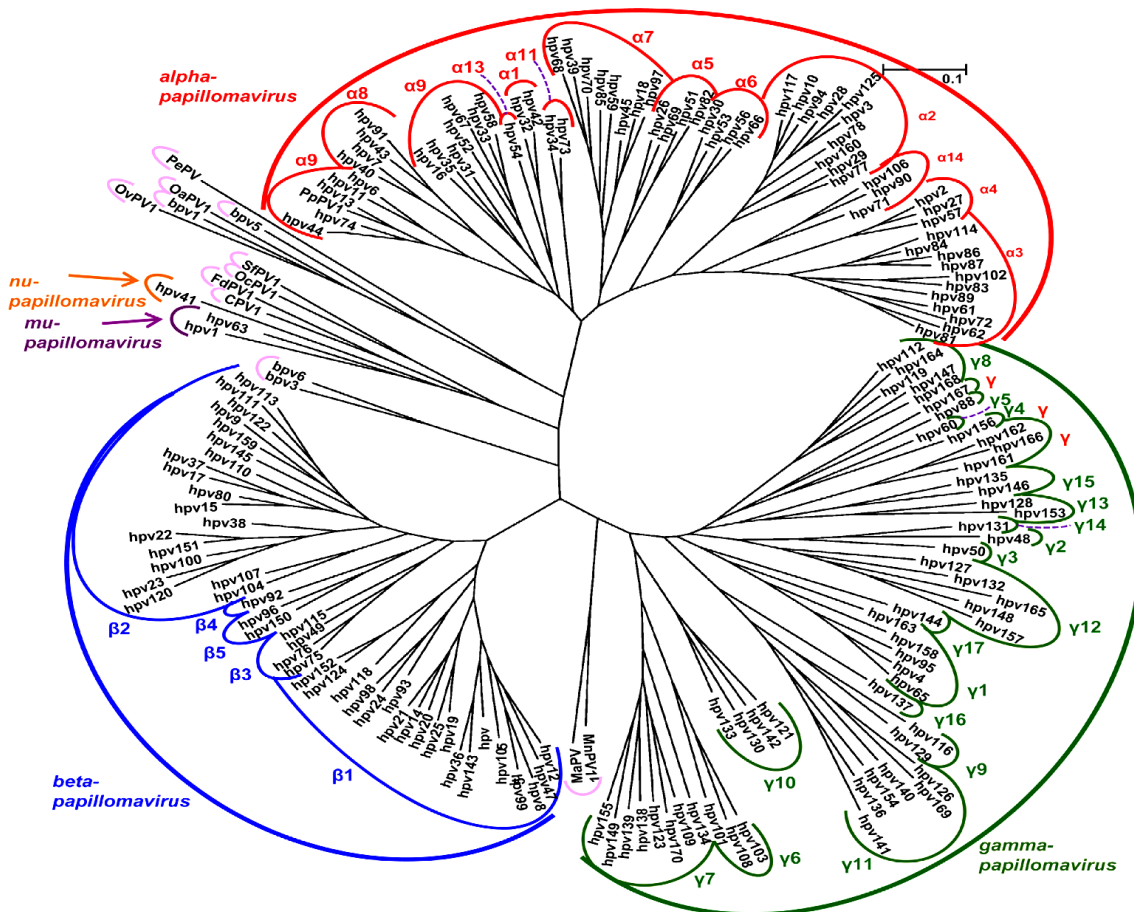


Figure 3. Classification phylogénétique des papillomavirus (de Villiers et al, 2004)

Historiquement, les HPV ont été classifiés en types, sous-types et/ou variants, mais cette classification n'est pas reconnue par le « *International Committee on Taxonomy of Viruses (ICTV)* » (Tableau 1) (Van Doorslaer et al., 2018). L'utilisation de la nomenclature par "types" est cependant très largement répandue (de Villiers et al., 2004; Bernard et al., 2006). Les différents types ou génotypes se retrouvent à l'intérieur des espèces définies par l'ICTV.

Tableau 1. Comparaison de la nomenclature des papillomavirus selon l'ICTV et celle communément utilisée

Nomenclature	Désignations communes	Désignation ICTV
Famille	Papillomavirus	Papillomaviridae
Genre	Alpha papillomavirus	Alphapapillomavirus
Espèce	Alpha papillomavirus-9	Human papillomavirus 16
Type/Souche :	Human papillomavirus 16, 31, 35 etc.	Human papillomavirus16, 31, 35 etc.
Type-espèce	Non applicable	Terme identifiant au HPV typique du genre ou espèce

Afin de fournir une classification robuste entre les différents génotypes, des études ont montré que la région non codante LCR fournit également suffisamment d'informations concernant la variabilité du génome des HPV pour servir de support à leur classification. Cette région est ainsi de plus en plus utilisée pour décrire la diversité intratype des HPV, en fournissant une classification robuste en lignées ou sous lignées (Cornet et al., 2012). Ainsi, l'analyse phylogénétique réalisée à partir de la région LCR de plusieurs isolats de HPV16 permet de regrouper ces variants génétiques en différentes lignées ou sous-types. La différence entre deux sous-types étant définie par une différence de plus de 5% dans la région LCR de deux isolats d'un même génotype (Calleja-Macias et al., 2005; Raiol et al., 2009).

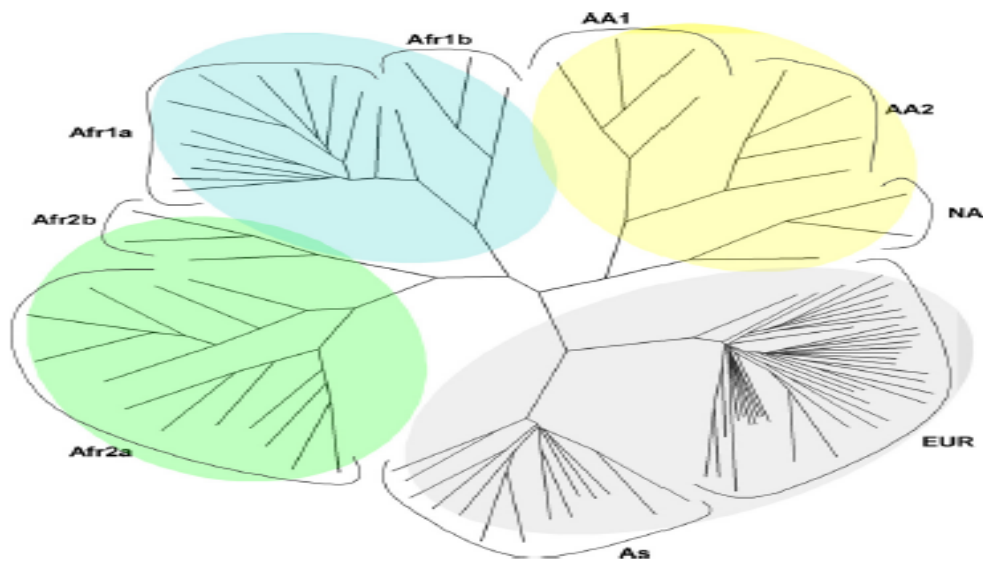


Figure 4. Arbre phylogénétique HPV16 basé sur 99 séquences E6/LCR (Cornet et al., 2012)

D'autres méthodes de classification alternatives, non-taxonomiques, se basant sur certains critères tels que le site anatomique préférentiel d'infection, le tropisme et le potentiel oncogène ont été élaborées. Selon le site d'infection, on distingue : les HPV cutanés et les HPV muqueux (de Villiers et al., 2004). Au niveau cutané, on trouve principalement des HPV appartenant aux genres bêta, gamma, mu et nu, principalement responsables des lésions bénignes telles que les verrues vulgaires (HPV1, 2 et 4) et impliqués dans le développement de certains cancers de la peau (de Villiers et al., 2004 ; Mboumba Bouassa et al., 2017). Au niveau des muqueuses, on trouve principalement des HPV appartenant au genre alpha. Ils peuvent être classés selon leur potentiel oncogène (Muñoz et al., 2003; Bouvard et al., 2009). On distingue les types à bas risque "low-risk" (LR)-HPV, (HPV6, HPV11,...) à l'origine d'affections bénignes (verrues, condylomes acuminés) et les types à haut risque "high-risk" (HR)-HPV, (HPV16, HPV18, -31, -33, -35, -45,...) causant les cancers (Muñoz et al., 2003; Bouvard et al., 2009).

II. Épidémiologie de l'infection génitale à HPV chez la femme en Afrique subsaharienne

Avant-propos : Cette partie est une synthèse de la littérature décrivant les principaux travaux réalisés et les principales découvertes associées à la compréhension de l'épidémiologie de l'infection à HPV en Afrique subsaharienne ainsi que l'état des lieux des stratégies mises en place pour juguler ce problème majeur de santé publique. Cette revue de la littérature intitulée : **“Cervical cancer in sub-Saharan Africa: a preventable noncommunicable disease”** a fait l'objet d'une publication dans le journal *“Expert review of anti-infective chemotherapy”*.

Résumé :

Introduction: Les infections causées par les papillomavirus humains à haut risque oncogène (HR-HPV) sont responsables de 7,7% des cancers dans les pays en développement, principalement le cancer du col utérin. Cette maladie est en augmentation constante en Afrique subsaharienne, avec plus de 75 000 nouveaux cas et 50 000 décès par an, aggravée par l'infection par le VIH.

Domaines couverts: L'état actuel de l'épidémiologie du cancer du col utérin associé aux HR-HPV en Afrique subsaharienne a été systématiquement révisé. Les principales questions abordées ici sont liées au fardeau du cancer du col utérin sur la santé publique en Afrique subsaharienne et aux prévisions pour les décennies à venir, notamment l'épidémiologie moléculaire et les déterminants de l'infection à HPV en Afrique, ainsi que les mesures de prévention prometteuses en cours d'évaluation en Afrique.

Commentaire d'expert: D'ici 2030, le cancer du col de l'utérus fera plus de 443 000 victimes chaque année dans le monde, dont la plupart en Afrique subsaharienne. L'augmentation de l'incidence du cancer du col utérin en Afrique pourrait contrecarrer les progrès réalisés par les femmes africaines dans la réduction de la mortalité maternelle et l'augmentation de la longévité féminine. Néanmoins, le cancer du col utérin est une maladie non transmissible potentiellement évitable, et des stratégies d'intervention visant à éliminer le cancer du col utérin en tant que problème de santé publique devraient être mises en œuvre de toute urgence.

REVIEW



Cervical cancer in sub-Saharan Africa: a preventable noncommunicable disease

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ABSTRACT

Introduction: Infections caused by high-risk human papillomavirus (HPV) are responsible for 7.7% of cancers in developing countries, mainly cervical cancer. This disease is steadily increasing in sub-Saharan Africa, with more than 75,000 new cases and 50,000 deaths yearly, further increased by HIV infection.

Areas covered: The current status of cervical cancer associated with HPV in sub-Saharan Africa has been systematically revised. The main issues discussed here are related to the public health burden of cervical cancer in sub-Saharan Africa and predictions for the coming decades, including molecular epidemiology and determinants of HPV infection in Africa, and promising prevention measures currently being evaluated in Africa.

Expert commentary: By the year 2030, cervical cancer will kill more than 443,000 women yearly worldwide, most of them in sub-Saharan Africa. The increase in the incidence of cervical cancer in Africa could counteract the progress made by African women in reducing maternal mortality and longevity. Nevertheless, cervical cancer is a potentially preventable noncommunicable disease, and intervention strategies to eliminate cervical cancer as a public health concern should be urgently implemented.

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1. Introduction

Human papillomavirus (HPV) infections are among the most widespread human viral infections worldwide that can infect the skin and mucous membranes. These mucosal tropic viruses are transmitted via skin-to-skin and sexual contact (including vaginal, anal, and oral sex) [1,2]. With an overall incidence of more than 30 million new cases per year, HPV infection is probably the most common viral sexually transmitted infection (STI) in the world [3]. Most women are exposed very early to HPV at first sexual intercourse [1]. Nearly 75% of all sexually active men and women will be infected with a type of HPV at some point during their lives [4]. Globally, about 12% of women with normal cytology show cervical HPV shedding detected by molecular biology, with the highest prevalence (24%) found among women in Sub-Saharan Africa in comparison with women from Eastern Europe (21%) or Latin America (16%) [5]. It is important to note that Sub-Saharan Africa has the highest prevalence of cervical HPV infection in the world, with some prevalence reported particularly high, such as in Guinea (48%) or Mozambique (41%) [6,7].

More than 170 HPV genotypes, numbered sequentially, have been identified [8]. Fifteen types are recognized as high-risk HPV (HR-HPV) and involved in HPV-associated cancers: types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82. It is widely recognized that persistent HR-HPV

infections are causally associated with anogenital cancers (particularly cervical cancer) and precursor lesions [9,10] (Figure 1). Globally, HPV-16 is the most prevalent type detected in HPV-associated cancers, followed by HPV-18. Together, HPV-16 and HPV-18 cause about 70% of cervical cancers worldwide [5]. The two most common low-risk mucosal HPV types are 6 and 11, which together cause about 90% of genital warts [11].

HR-HPV is responsible for 5.2% of all cancers worldwide, 2.2% for cancers in developed countries, and 7.7% in developing countries [19]. Cervical cancer is caused by HR-HPV infections of the exocervical mucosa [9]. After a period of silent incubation for more than 10–20 years, a transformation of the squamous epithelial cells (atypical squamous cells of undermined significance) or glandular cells (atypical glandular cells) occurs and finally leads to invasive cervical cancer (ICC) [1,20,21]. At that stage, the disease is a rapidly evolving disease with a fatal prognosis when diagnosed too late, causing an inevitable and inhuman death when there is no surgical management and palliative care. With an estimated global prevalence of 2.3 million women, a global annual incidence of 528,000 new cases, and an estimated mortality of 266,000 deaths each year, cervical cancer is the second largest cancer involving

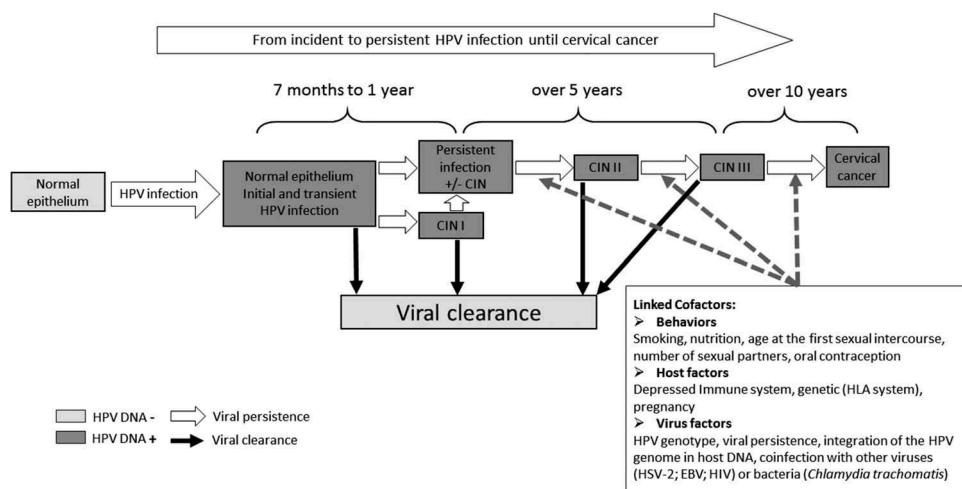


Figure 1. Course of HPV infections, cervical lesions and prevention strategies. In 90% of cases the infection is transient and naturally eliminates by the host immune system within one to two years after sexual contamination. But in few cases transforming events can lead to neoplasia beginning with the linearization of the circular viral genome by the viral DNA cleavage within the E2 gene and following by its integration into the host genome: it's the persistent viral infection. The integration of the HPV viral DNA interrupts the downregulation of the oncogenes E6 and E7 and promotes the inhibition of the pro-apoptotic p53 and pRB proteins. The p53 and pRB proteins play key roles in cell cycle control and cellular DNA repair. Their inactivation by E6 and E7 proteins leads to HPV-induced cell transformation. The neoplastic transforming capacity of E6 and E7 is greatest for high-risk viral types, particularly HPV types 16 and 18. The integration of the viral genome into the host DNA leads to the persistence of the virus and can lead to progressive lesions such as cervical intraepithelial neoplasia (CIN 1, CIN 2 and CIN 3) in the mucosa of the cervix. Precancerous lesions can develop into cancer 10–15 years after infection with the virus [12,13]. However, the long incubation period of HPV infection and the fact that an infected woman does not systematically develop cervical cancer suggests the involvement of associated cofactors participating in the process of carcinogenesis. Potential HPV cofactors include smoking [14], pluriparity [15], long duration of oral contraceptive use [16], and other sexually transmitted infections such as HIV, HSV-2 or *Chlamydia trachomatis* infection [17,18].

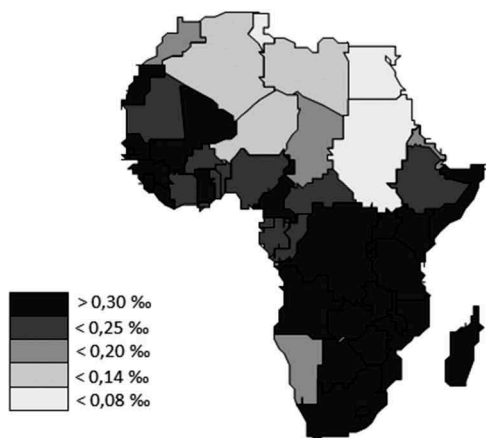
women worldwide [7]. Every minute of every day, a new case of cervical cancer strikes a woman [7].

2. Cervical cancer in Sub-Saharan Africa: an economical, socio-demographical, and public health concern

Cervical cancer is the most common cancer in women in many countries in Sub-Saharan Africa, with more than 75,000 new

cases and nearly 50,000 deaths per year [6,22] (Figure 2). For example, the annual incidence rate of cervical cancer is about 75.9 cases per 100,000 women in Malawi [23]. Each year in Africa, cervical cancer affects 27.6 per 100,000 women, of whom 17.5 per 100,000 die from it [7]. By comparison, the incidence of cervical cancer in the United States among the African-American population was about 70 per 100,000 women in the 1960s, when the first cervical screening smears were introduced, against currently about 6 new cases per 100,000 women [6,7].

a. Cervical cancer incidence in Africa (2012)



b. Estimated number of new cervical cancer cases in 2012 and 2030

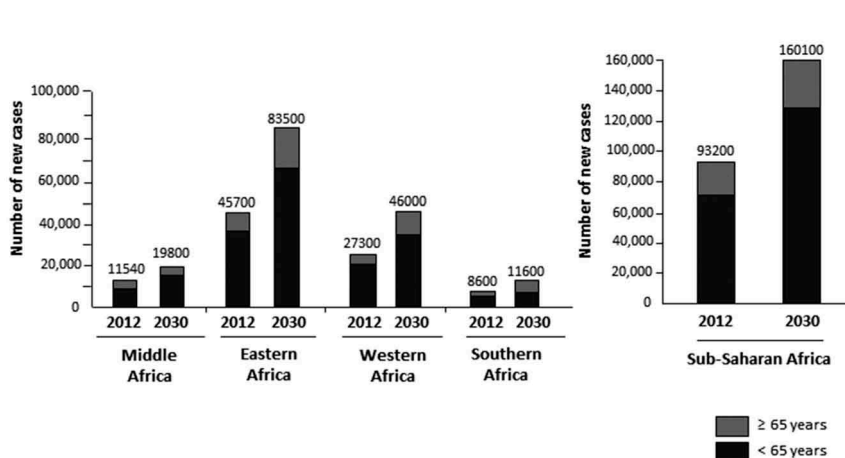


Figure 2. Burden of cervical cancer in Africa. a. Estimated incidence of cervical cancer in 2012 [7]; b. Estimation and evolution in the number of new cases of cervical cancer between 2012 and 2030 according to the age (from GLOBOCAN 2012) [6]. The map shows the age-standardized cervical cancer incidence rate across the globe. The regions shown in dark green have got very low incidence rates of cervical cancer with less than 9.3 cases per 100,000 women. In contrast, the countries shown in dark red have incidence rates which between 35.8 and 93 per 100,000. Thus women living in much of sub-Saharan Africa are almost 10 more likely to develop cervical cancer than a woman living in the US.

Commonly qualified as noncommunicable disease, cervical cancer has been for a long time under-prioritized in developing countries especially in Sub-Saharan Africa [24,25]. In this region of the world, HPV infection and cervical cancer have become particularly worrying in terms of socio-demographic, economic, and health conditions.

At the economic level, the impact of cervical cancer is greater in resource-limited countries as in developed countries. Thus, among all cancers, cervical cancer is responsible for nearly 2.4 million years of life lost among women between the ages of 15 and 59, which represents 22% of the years of life in countries with limited resources, 10% in middle-income countries, and 5% in developed countries [26].

According to the World Health Organization (WHO), cervical cancer will kill more than 443,000 women a year worldwide by 2030 [27] (Figure 3). More than 98% of these deaths will occur in developing countries, most of them in Sub-Saharan Africa [7]. The increase in the incidence of cervical cancer in the world in general and particularly in Africa is annihilating the progress made in African women's survival and longevity. Thus, the decline of almost 45% in maternal mortality over the last 20 years in Africa [28] and the increased life expectancy [6] (Figure 3) have now paradoxically become simultaneous with the increase in female mortality in Africa, due to cervical cancer and the high prevalence of female HR-HPV carriage.

Immigrant women from Sub-Saharan Africa living in metropolitan France, especially the first-generation women with prevalence of genital HR-HPV infection, are reflecting the infectious epidemiology of African endemic areas. As such, they could constitute a vulnerable population, including a high prevalence of cervical HPV infection and the presence of viral cofactors such as HIV and Herpes simplex virus 2 (HSV-2) infections.

3. HPV prevalence and genotype distribution in Sub-Saharan African women

Data on the worldwide prevalence and the type of distribution of HPV mainly derived from studies conducted in Europe, North America, Asia, and Latin America [30]. For the implementation of effective vaccination programs and appropriate epidemiological monitoring of viral ecology before and after vaccination in Sub-Saharan African setting, knowledge on HPV prevalence and HR-HPV genotype distribution is highly

essential. Since the last decade, epidemiological studies have been conducted in several Sub-Saharan African countries.

In 2010, Bruni et al. [5] have published the results of a global meta-analysis including 194 studies conducted between 1995 and 2009 on 1 million women (7705 women from Africa) with normal cytology in several sites in five continents in order to make a comprehensive assessment of the HPV burden among women worldwide. In this study, the global HPV prevalence was 11.7% (95% confidence interval [CI] 11.6–11.7%). Particularly, HPV prevalence in Sub-Saharan Africa (24.0%) was higher than in any other area. Moreover, adjusted HPV prevalence among Sub-Saharan African women was estimated at 33.6%, 19.6%, and 17.4% in Eastern, Western, and Southern Africa, respectively. The five most common HPV genotypes worldwide were HPV-16 (3.2%), HPV-18 (1.4%), HPV-52 (0.9%), HPV-31 (0.8%), and HPV-58 (0.7%) [5].

Likewise, the review performed by De Vuyst et al. [6] showed large variations in HPV prevalence among women with normal cytology across Sub-Saharan African region ranged between 3.2% in Sudan and 47.9% in Guinea. This included Western Africa where the highest HPV prevalence (47.9% in Guinea) was found, whereas the lowest rates were in North-Eastern Africa (3.2% in Sudan). In Southern Africa, women with normal cytology showed HPV prevalence ranged from 15.5% to 20.4%, and in Middle Africa, especially from Democratic Republic of Congo, the HPV prevalence was 8.7% in women with normal cytology. Remarkably, as found in Mozambique (41.4%), women with normal cytology in South-Eastern Africa showed some of the highest HPV prevalence in Sub-Saharan Africa. In this overview, data from Western Africa reveal the predominance of HPV-16 (6.6%) followed by types 45 (4.7%), 52 (4.0%), and 18, 35, and 58 (3.2%) [6].

In Addition, Ogembo et al. [30] have performed a systematic review and meta-analysis including 71 studies from 23 African countries to achieve a comprehensive assessment of the overall prevalence and distribution of HPV genotypes in African women with and without different cervical neoplasia. Overall prevalence of HPV-16/-18 was 4.4% and 2.8% of women with normal cytology, 12.0% and 4.4% with atypical cells of undetermined significance, 14.5% and 10.0% with low-grade squamous intraepithelial lesion, 31.2% and 13.9% with high-grade squamous intraepithelial lesion (HSIL), and 49.7% and 18.0% with ICC, respectively. These results showed that HPV-16/-18 account for 67.7% of ICC cases among African women and are quite similar to the percentage (70%) reported for genotypes HPV-16/-18 worldwide [31,32].

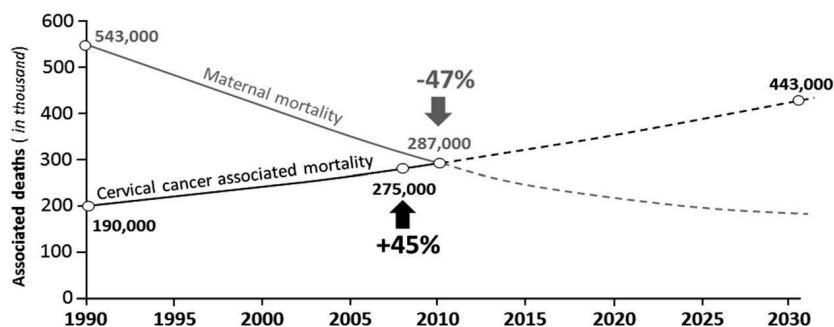


Figure 3. Evolution and forecasting of maternal and cervical cancer-related mortalities between 1990 and 2030 in the world. Since the 2010s, the mortality associated to cervical cancer is become more important that maternal mortality; in 2030, the predicted mortality due to cervical cancer will have largely eradicated the decline in maternal mortality observed since the 1990s [29].

Many other studies conducted during these last 6 years showed fairly similar deductions on the HPV prevalence in different regions of Sub-Saharan Africa and the distribution of different HPV genotypes in women with or without normal cytology. In Eastern Africa, Watson-Jones et al. [33] have reported 74% of HPV prevalence among 142 sexually active healthy young African females recruited for a safety and immunogenicity trial of the bivalent HPV-16/18 vaccine in Tanzania. The most prevalent HPV genotype infections were HPV-45 (16%), HPV-53 (14%), HPV-16 (13%), and HPV-58 (13%). These results corroborate that HPV was highly prevalent and acquired soon after sexual debut. HPV vaccination should be administered very early in puberty optimally before sexual debut [32]. In Southern Africa, among 224 young women, a study has registered an overall HPV prevalence of 76.3%. Also, here, the large-scale delivery of HPV vaccine should be prioritized to prevent HPV acquisition and reduce HPV-related morbidity [34].

In 2012, McDonald et al. [35] have designed a large study on 8050 HIV-negative women to describe the profile of HR-HPV genotypes among women population in Sub-Saharan Africa. The overall HR-HPV prevalence was 20.7%. HPV-16, HPV-35, and HPV-45 were the leading contributors to cervical intraepithelial neoplasia (CIN) 2, 3 [34]. Another study performed by Akarolo-Anthony et al. [36] on 278 HIV-infected and uninfected women in Nigeria showed a relatively different HPV genotypes distribution. Indeed, among HIV-infected women, HPV-35 (8.7%) and HPV-56 (7.4%) were the most prevalent HR-HPV, while HPV-52 and HPV-68 (2.8% each) were the most prevalent HR-HPV types among HIV-uninfected women [36].

The study carried out by Boumba et al. [37] in 125 HSIL and ICC cases from Congolese women has shown an overall HPV prevalence of 89.6%, and HPV-16, HPV-33, HPV-18, and HPV-31 were the most common genotypes found in women with HSIL and ICC [37]. Another study carried out in Middle Africa, in Cameroun, on 181 women with ICC has also reported high HPV prevalence ranged from 45.1% for women with multiple HPV genotypes to 54.9% with single HPV genotype infection. The HPV genotype distribution was also quite similar to that reported previously. In Gabon, Zoa Assoumou et al. [38] have reported an overall HPV prevalence of 60% in the women population, including 57.5% and 76.9% for women with normal and abnormal cytology, respectively. Another study carried out by Moussavou Boundzanga et al. [39] in Gabonese women showing abnormalities observed upon VIA/VILI highlights the high prevalence (82.9%) of HPV infection by molecular analysis. Similarly, to those reported previously, the main HPV types found in this study were HPV-16, HPV-18, and HPV-33, and almost the half (42.3%) of these women harbored multiple HPV infections.

4. Impact of HIV on the epidemiology of cervical cancer in Sub-Saharan Africa

Despite the use of combination antiretroviral therapy (cART), the burden of HIV epidemic has negatively impacted the health of Sub-Saharan African people. According to the WHO, more than 36.9 million people worldwide were living

with HIV at the end of 2014. Moreover, 25.8 million of them were living in Sub-Saharan Africa among which 59% are women [40]. Likewise, cervical cancer is the leading cause of cancer death in women in Sub-Saharan Africa [22]. Both HPV and HIV infections share the same route of transmission, and cervical cancer is since 1993 considered as an AIDS-related disease. Indeed, HPV is commonly known as a risk factor associated with HIV acquisition. Likewise, HIV infection is associated with an increased risk of acquiring new HPV infections and the reduction in the rate of HPV clearance [41,42].

These two last decades have been marked by the introduction and the widespread use of combination antiretroviral treatment in Sub-Saharan Africa with a correlated increase in life expectancy of HIV-infected women. De Vuyst et al. [43] performed a review on the impact of cART on the development of cervical cancer in HIV-infected women. This study suggests that cART seems to have a beneficial effect on the evolution of precancerous lesions in HIV-positive women, with a much longer life expectancy than those infected by HIV but untreated. Therefore, cART might finally expose them to develop cervical cancer. Unfortunately, the progress gradually made for nearly two decades by international public health policies with the Millennium Development Goals (MDG) in improving maternal health and reducing AIDS-related death (MDG Nos. 5 and 6) and more recently with the Sustained Development Goals (SDG Nos. 3.1 and 3.3) are annihilated by cervical cancer mortality among African women. The significant decline of almost 45% in maternal mortality for more than 20 years in Africa [28] has been now paradoxically annihilated by the increase in female mortality because of cervical cancer in Sub-Saharan Africa, due to the high prevalence of female HR-HPV carriage and the increased life expectancy [6] (Figure 3).

5. Cervical cancer screening in Sub-Saharan Africa

5.1. Cytology for cervical cancer screening

Cervical cancer is an emergent and potentially preventable noncommunicable disease. This disease benefits from the possibility of a primary prevention with the vaccination, a secondary prevention by the early diagnosis of situations at risk, and tertiary prevention by the early diagnosis and treatment of proven cases of cervical neoplasia (Table 1). Few noncommunicable diseases benefit from such a wide range of prevention and management tools, and the ultimate and often fatal form of the disease, the ICC, should no longer occur. The implementation and sustainability of prevention programs against HPV infection and cervical cancer have proved their efficacy in developed countries. Indeed, in several of those countries well-structured preventive strategies against HPV infection have allowed to reach a great decline in cervical cancer incidence and mortality [44]. Note that, since the introduction of Pap smear-based screening in the United States, the incidence rate of cervical cancer has dramatically decreased by over 50% and 70%, respectively, among white and African-American women [45]. The notable efficacy of prevention programs against cervical cancer in developed countries contrasts with the impotence of the cervical cancer

Table 1. Primary, secondary, and tertiary preventive strategies according to age groups.

Target population	Primary prevention ^a	Secondary prevention ^b	Tertiary prevention ^c
	Girls: 9–14 years	Women: >30 years of age	Women with invasive cervical carcinoma (generally > 45 years)
Preventive strategies	<ul style="list-style-type: none"> • HPV vaccination: 9–14 years • Health education and services: ≥ 10 years • Sexual health education according to the age group • Promote the condoms use • Preventing tobacco use and support for cessation 	Screening and treatment <ul style="list-style-type: none"> • ‘Screen and treat’ with low-cost point-of-care rapid HPV testing technology combined with VIA • With low-cost point-of-care rapid HPV testing technology combined with colposcopy for those positive followed by cryotherapy 	<ul style="list-style-type: none"> • Surgery • Radiotherapy • Chemotherapy • Palliative care

HPV: human papillomavirus.

^aAll strategies put in place to prevent HPV infection in people who are not yet infected.

^bAll measures aimed to identifying people at risk (those with persistent HR-HPV infection), detecting precancerous lesions at their earliest stage, and applying prompt and effective treatment to stop their development in invasive cervical cancer.

^cActions aimed to reduce the progression and complications of proven cervical cancer. It consists to reduce disabilities and disadvantages (pain) and to improve the quality of life of people with terminal cancer.

control policies in many developing countries. In fact, the worsening epidemiological situation of cervical cancer in Sub-Saharan Africa is largely the result of the lack of coherent national programs for the prevention and management of cervical cancer, although limited experiments demonstrate the feasibility [46]. The difficulties of implementing and sustaining such strategies are diverse. One of the main barriers compromising the epidemiological control of cervical cancer in Sub-Saharan Africa is the fact that cervical cancer has been under-prioritized for a longtime compared to the leading widespread communicable diseases in Sub-Saharan Africa such as HIV, tuberculosis, and malaria [46]. Another major handicap faced by these programs is that secondary prevention based on Pap smear is found to be unsuitable for the Sub-Saharan African setting. For the effectiveness of cytology-based screening, the Pap smear must be followed by colposcopy and histological assessment for women with abnormal smears and finally by surgical treatment for histologically proven precancerous lesions. This screening process requires a too long waiting period for the return of the results. Thus, a lot of women do not return retrieved conclusions of their cytological examination. This examination process is too expensive for most of women in Sub-Saharan African countries. Furthermore, it should be repeated at least once every 2 or 3 years [47]. Added to this, adequate health infrastructures that can provide various prevention programs and support strategies are struggling to sprout or are almost nonexistent in some countries. The few facilities equipped and empowered to ensure adequate cervical cancer prevention are located, where they exist, in large cities. But more than 50% of women in most of Sub-Saharan African countries live at more than 10 km from the nearest primary care center [31,48]. Thus, the access to these structures is unfortunately limited to urban populations. So, there is a selection bias making that cancer registries in many Sub-Saharan African countries are not representative of the entire population of these countries. This weakens the coverage of the prevention and awareness programs against cervical cancer on the most at-risk population, making them therefore ineffective [46].

Finally, high-quality cytology-based screening requires highly trained and qualified medical staff for adequate interpretation of cervical smears, colposcopy, and histological assessment. But in most of Sub-Saharan African countries, which have the highest cervical cancer incidence rates, these medical specialists are lacking [31,46].

In these recent decades, alternative approaches to cytology-based screening which would overcome many barriers identified in most of the low- and middle-income countries have been actively evaluated in several developing areas around the world. However, fewer of these studies have been carried out in Sub-Saharan African countries [49]. Relatively, the two most widely studied alternative approaches to cervical cancer prevention are visual inspection methods and HPV DNA testing [49,50].

Arbyn et al. [51] have performed the largest cross-sectional study ever done in the low-resource settings, on 58,000 women to evaluate the performance of current worldwide screening methods in India and Sub-Saharan Africa. In this study, five screening methods including visual inspection of the cervix uteri after application of diluted acetic acid (visual inspection with acetic acid or ‘VIA’), or Lugol’s iodine (visual inspection with Lugol’s iodine or ‘VILI’) or with magnifying device (visual inspection with acetic acid and with magnification or ‘VIAM’), the Pap smear, and Hybrid Capture 2 (HC2) assay (Digene, Gaithersburg, MD, USA) as HPV DNA testing were evaluated. VIA showed a sensitivity of 79% (95% CI 73–85%) and 83% (95% CI 77–89%), a specificity of 85% (95% CI 81–89%) and 84% (95% CI 80–88%) for the outcomes CIN2+ and CIN3+, respectively. VILI was on average 10% more sensitive (91%; 95% CI 87–94% for CIN2+ and 94%; 95% CI 91–97% for CIN3+) and equally specific. VIAM showed similar results as VIA. The Pap smear showed lowest sensitivity 57% (95% CI 38–76%) for CIN2+ but the specificity 93% (95% CI 89–97%) was rather high. The HC2 assay showed a surprisingly low sensitivity for CIN2+ of 62% (95% CI 56–68%) and a specificity of 94% (95% CI 92–95%). Although there is the possibility of an overestimation of both sensitivity and specificity of VIA and VILI, the major finding in this study is still the

consistently higher but equal specificity of VILI compared to VIA. Overall, visual inspection methods were more sensitive than the others [51].

Fokom-Domgue et al. [52] have performed a systematic review and meta-analysis of 15 diagnostic test accuracy studies carried out in Sub-Saharan African countries in 2015. The aim of this study was to assess and compare the accuracy of VIA, VILI, and HPV DNA testing alternative stand-alone methods for primary cervical cancer screening in Sub-Saharan Africa. For each screening test, the number of tested women was 61,381 for VIA, 46,435 for VILI, and 11,322 for HPV DNA testing. Prevalence of CIN2+ did not vary by screening test and ranged from 2.3% (95% CI 1.5–3.3%) in VILI studies to 4.9% (95% CI 2.7–7.8%) in HPV DNA testing studies. Positivity rates of VILI, VIA, and HPV DNA testing were 16.5% (95% CI 9.8–24.7%), 16.8% (95% CI 11.0–23.6%), and 25.8% (95% CI 17.4–35.3%), respectively. Pooled sensitivity 95.1% (95% CI 90.1–97.7%) was higher for VILI than VIA 82.4% (95% CI 76.3–87.3%) in studies where the reference test was performed in all women ($P < 0.001$). Pooled specificity of VILI 87.2% (95% CI 78.1–92.8%) and VIA 87.4% (95% CI 77.1–93.4%) were similar ($P = 0.85$). Pooled sensitivity and specificity were similar for HPV DNA testing versus VIA (both $P \geq 0.23$) and versus VILI ($P \geq 0.16$). Accuracy of VILI and VIA increased with sample size and time period. Taken together, data from this study show that both visual inspection methods of the cervix uteri and HPV DNA testing-based screening are simple and affordable alternative to cytology, particularly VILI which demonstrates here higher sensitivity than VIA [52].

5.2. Visual inspection techniques for cervical cancer screening

Visual inspection with acetic acid and/or Lugol's iodine has been shown to be good alternative to cytology-based screening of precancerous lesions. Indeed, this method has been shown to have adequate sensitivity, quite similar to cytology (Table 2). Despite its lower specificity compared to cytology, visual inspection (VIA and/or VILI) requires fewer resources and is technologically more accessible by primary care staff than cytology. Also, quality assurance for VIA is challenging in Sub-Saharan Africa because of frequent lack of staff trained for test providers; constant monitoring and frequent re-training of test providers are needed to ensure consistency in performance and sustain the quality of the interpretation of tests.

The success of cytology screening-based prevention is largely due to the regularity of screening visits (once or twice every 2 or 3 years). However, the time between screening and the return of results and the treatment of precancerous lesions are major barriers, which can explain the failure of this prevention strategy in most of developing countries. Well beyond the time for results, the quality of the diagnosis provided by the Pap test remains a major asset for this method of screening [46]. However, with the visual inspection-based screening (VIA/VILI), the results can be communicated to the patient immediately. Furthermore, in a 'screen and treat' strategy, when ambulatory treatment is available, the treatment can be provided during the same visit. But, a limitation of visual inspection methods is that they are relatively not reliable in postmenopausal women because of changes that occur in the mucosa of the cervix at this age [47].

Despite its limitations, advantages such as single visit in 'screen and treat' approach, its low cost and its simplicity make the visual inspection method the only reasonable alternative to cytology in many developing countries in the Sub-Saharan African setting. However, once visual inspection methods become successfully implemented in these countries, it should be relatively easy to introduce more sensitive methods of screening such as HPV DNA testing. Currently, this screen and treat approach may significantly increase the effectiveness of prevention programs and reduce morbidity and mortality associated with cervical cancer in the Sub-Saharan African settings [55].

5.3. HPV testing technologies

Another alternative to cytology already used for primary screening in several countries [53] is the HPV DNA testing method. Indeed, understanding the role of HPV infection in the etiology of cervical cancer and the constant innovation in the field of molecular biology have created nearly 30 years ago new opportunities for the prevention of cervical cancer such as molecular tests for detecting HR-HPV genotypes in cervical swabs. Many of these tests are available for routine laboratory service in many developed countries. HPV DNA testing is an excellent alternative to cytology for cervical cancer screening because it is a higher sensitive (Table 2) and reproducible laboratory technique than cytology [53].

However, if visual inspection is not recommended for the postmenopausal women, HPV DNA testing is not recommended for women under the age of 30 years. Indeed, HPV infection in this

Table 2. Screening tests for secondary prevention of human papillomavirus (HPV) genital infections in women [53].

Characteristics	Visual inspection tests		Cytology	HPV DNA molecular tests
	Visual inspection with acetic acid (VIA)	Visual inspection with Lugol's iodine (VILI)		
Sensitivity	67–79%	78–98%	47–62%	82–100%
Specificity	49–86%	73–91%	60–95%	75–96%
Number of visits required for screening and treatment	<ul style="list-style-type: none"> • Single-visit or 'screen and treat' approach where outpatient is available • At least two visits if outpatient is not available 		≥2 visits	≥2 visits
Comments	Assessed over the last decade in many settings in developing countries	Assessed by the International Agency for Research on Cancer over the last 5 years in India, Burkina Faso, Congo, Guinea, Mali, and Niger [54].	Assessed over the last 50 years in a wide range of settings in developed and developing countries	Assessed over the last decade in many settings in developed and relatively few in developing countries

group of women is common, and most of these infections are likely to be transient with a low likelihood of developing into cancer (Table 1). For many countries in Sub-Saharan Africa, commercially available HPV DNA tests such as Hybrid Capture 2 (Digene, Gaithersburg, MD, USA), one of the most commonly used tests, are still more expensive. These tests require also sophisticated processing in the laboratory, and the deadline for the results is long enough. Although HPV DNA testing shows many advantages compared to other screening methods, these techniques are still relatively non-suitable for the Sub-Saharan African settings. Several studies have reported that new rapid HPV DNA tests, such as *careHPV* (Qiagen, Gaithersburg, MD, USA) or Cepheid Xpert® HPV (Cepheid Inc., Sunnyvale, CA, USA), are being developed and evaluated for developing countries [56–58]. Recently, the *careHPV* has been evaluated in Burkina Faso and South Africa among HIV-1-infected women, and it has been found to be a reliable tool for cervical cancer screening in Sub-Saharan African settings and particularly in HIV-1-infected women [59]. The *careHPV* has a lower cost per test than Hybrid Capture 2, and it is simpler to carry out. Furthermore, it is portable and allows for field interpretation results within 2.5 h. This test shows to have a relatively good sensitivity 83.3% [57]. Moreover, with HPV DNA testing, specimen can be obtained by self-collection, with relatively complete preservation of the sensitivity and specificity of the screening method. Self-collection, which can be done at home, is relatively good accepted by women [60]. The rapid turn-around time (within 2 h) of the Cepheid Xpert® HPV system facilitates a ‘see and treat’ approach and provides partial genotyping data (allowing assessment of risk beyond HPV-16/-18), making this point-of-care technology a relevant contender for routine use in low- and middle-income countries. [58]. Another very promising test for developing countries is the Aptima HPV assay from Hologic laboratories (Hologic, San Diego, CA, USA) [61]. The Aptima HPV assay identifies HR-HPV infections by targeting oncogenes E6/E7 mRNA, one of the most important markers to identify the presence of activity of a HR-HPV infection [61]. This assay makes possible to differentiate transient infections from persistent infections, thus identifying women with a higher risk of developing cervical cancer [62,63]. However, this assay requires the acquisition of sometimes-expensive analyzers and laboratory facilities. Despite its limitations, it is yet used in a limited number of Sub-Saharan reference laboratories, such as in South Africa [64].

Conventional and liquid-based cytology is generally affordable with prices ranging from 4 to 6 US\$, but it may be poorly available because of possible lack of cytologists. Molecular biology for HPV detection becomes an interesting alternative to cytology, although it requires the acquisition of sometimes-expensive analyzers and laboratory facilities. Nevertheless, the costs of reagents for HPV molecular biology have dramatically decreased ranging for example from 2 US\$ for *careHPV* [65] to 16 US\$ for Xpert® HPV [66].

6. Integrating cervical cancer screening into HIV existing services in Sub-Saharan Africa

Prevention and management of genital HPV infections in African women are nonetheless benefiting from AIDS programs. The United Nations General Policy Statement of 2016 on HIV and AIDS [67] and UNAIDS 2016–2021 Planning [68]

emphasize the importance of considering linkages HIV infection and HPV infections, the risk of development of cervical cancer in HIV-infected women, and the need to integrate prevention, screening, and management of cervical cancer into existing AIDS programs and services [69,70]. Cervical cancer is now considered a ‘preventable’ disease, and its screening and management are indirect objectives that must be integrated into the UNAIDS initiative 90-90-90. Thus, cervical cancer control is a medium-term target of UNAIDS, which specifies that every woman infected with HIV should be screened for cervical cancer by 2020, that HIV-negative women should be screened again within 3 years, and that mortality associated with invasive cervical neoplasia should be reduced by at least 25% by 2025 [71]. Programs for the prevention and early detection of cervical cancer in African women can also be potentially integrated into routine services for maternal and child health, and sexual and reproductive health program in line with the WHO, particularly targeted at vulnerable populations such as HIV-infected women and sex workers [72,73]. It should be noted that the effects of antiretroviral therapy on chronic infection and the risk of developing precancerous lesions and cancer are significantly developed in HIV-positive African women [74]. This reinforces the need for integrating cervical cancer prevention among HIV-positive women into HIV care services in Sub-Saharan African settings.

The prevention and early treatment of cervical cancer for countries with limited resources is supported by expert’s recommendations based on the latest published knowledge and experiences gained from medical and sociocultural personnel [75], with the specificities of the genital HR-HPV infections.

7. Vaccination against HPV infection to prevent cervical cancer in Sub-Saharan Africa

In the field of primary prevention, genital HPV infections and their consequences can be prevented by vaccination. The compatibility between the existing prophylactic vaccines spectrum and the epidemiology of the main types of HR-HPV circulating in Sub-Saharan Africa among women with normal cytology but also those involved in cervical cancer cases and precursor lesions argue to recommend the administration of available existing prophylactic vaccines bivalent Cervarix® (GSK Biologicals, Rixensart, Belgium) and quadrivalent Gardasil® 4 (Merck & Co. Inc., NJ, USA) to younger women mostly not infected yet with HPV-16/-18 and the strengthening of the efforts made in the screening of HR-HPV to prevent cervical cancer. [30]. Gardasil® 4 also protects against genotypes 6 and 11, which cause condylomas and recurrent respiratory papillomatosis. Both vaccines have been tested in randomized placebo-controlled trials and have been shown to be safe, immunogenic, and highly efficacious longtime after vaccination. These vaccines are more effective when administered before the first sexual intercourse and the acquisition of HPV infection [76]. Therefore, it seems legitimate to recommend HPV vaccination among adolescent girls, including HIV-positive women, who are at high risk to develop cervical cancer [77].

Otherwise, the HPV genotypes distribution is heterogeneous by region, resulting in relatively different prevalence

of HR-HPV genotypes. Thus, with the significant involvement of other genotypes such as the genotypes 31, 33, 35, 45, 52, 56, and 68 in cervical cancer and high-grade lesions, there is a critical need to broaden the spectrum of genotypes targeted by vaccination strategies implemented in Sub-Saharan Africa generally and particularly in Middle Africa. The new generation nonavalent Gardasil® 9 vaccine is effective against genotypes 6, 11, 16, 18, 31, 33, 45, 52, and 58. It would then protect against more than 90% of HR-HPV circulating genotypes in Sub-Saharan Africa (Figure 4(a)) [78]. The still high price of HPV vaccines from the private market is a brake on their use, but this is progressively lifted by international immunization programs with the GAVI or PATH financial support, making HPV vaccines cost affordable for several African national immunization programs (Figure 5).

In most of developed countries, the implementation of robust cytology-based screening for more than 40 years has been shown with decreasing rates of cervical cancer but most high-income countries are seeing a plateauing effect for more than 10 years explaining the need for vaccine [6]. More recently, the development and the widespread use of two prophylactic vaccines against HPV infection have considerably curbed the burden of cervical cancer in these countries [79]. The immune response against HPV types 16 and 18 (accounting for 70% of cervical cancer worldwide) induced by the vaccination with the two first-generation vaccines (bivalent and tetravalent vaccine) provides some cross-protection against types 45 and 31, both important in the worldwide etiology of cervical cancer, thus increasing the projected protection from vaccination to 75–80%. This induced cross-immunity remains, however, less

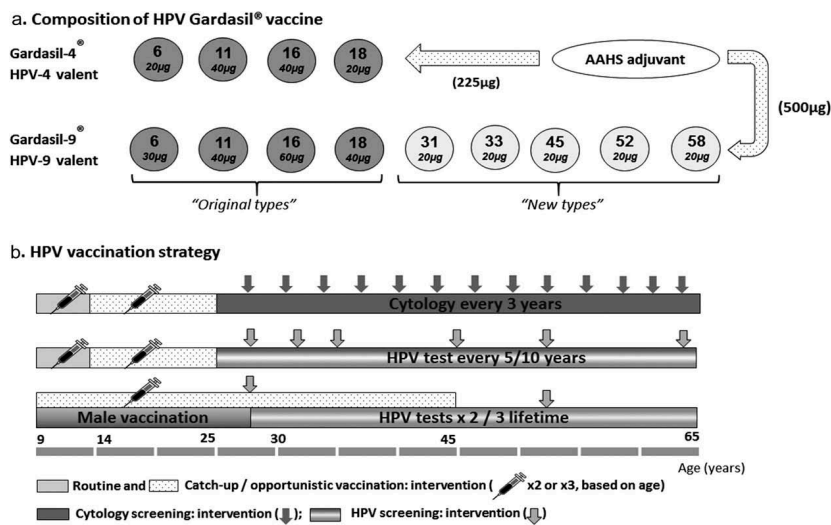
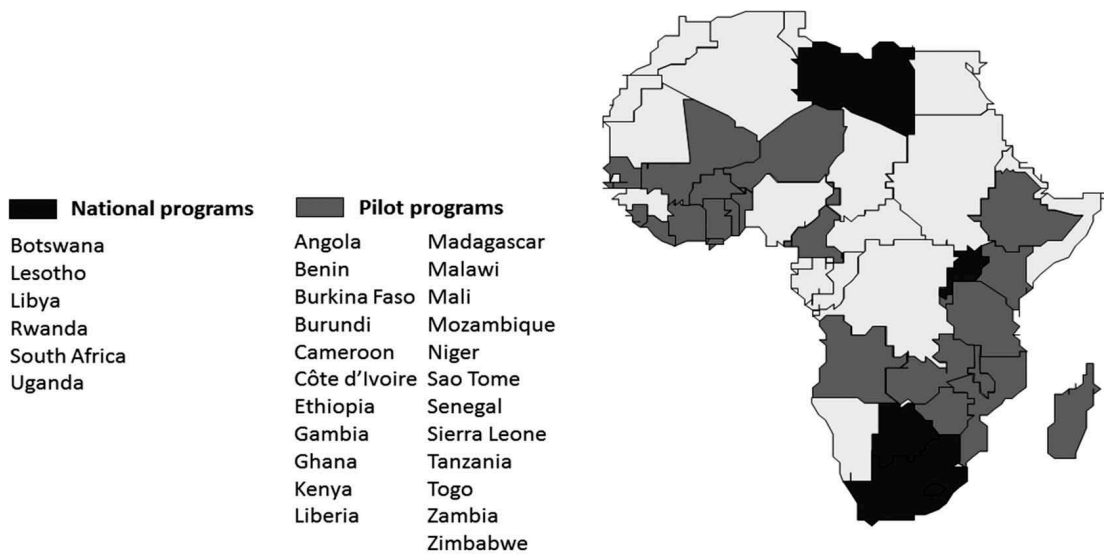


Figure 4. HPV vaccination in cervical cancer prevention. a. The composition of 4-valent and 9-valent HPV Gardasil® vaccine; b. The current HPV vaccination and cervical cancer screening strategy in developed countries and proposed FASTER initiative.



Source: Cervical Cancer Action

Figure 5. African progress in HPV vaccination. Map of African countries which had in 2016, introduced HPV vaccine in their national health program and those where HPV vaccine program is implemented through pilot program. This map has been adapted from HPV vaccine map: Global Progress in HPV Vaccination, status: November 2016. Available on Cervical Cancer Action at www.cervicalcanceraction.org.

Source: Cervical Cancer Action.

protective than the 9valent vaccine, thus reinforcing the need to wide spreading the use of the new generation of the broader spectrum 9valent vaccine in order to extend the protection against HPV infection. However, clinical evaluation-related data of these vaccines excluded, few years ago, Sub-Saharan African populations [50,80].

Since the last decade, at least 110 countries have licensed Cervarix® bivalent HPV vaccine and over 127 countries around the world have licensed the Gardasil® 4 vaccine [81]. In this number, the account of Sub-Saharan African countries is strongly increasing (Table 3). However, to date very few of them have currently introduced HPV vaccines into their national immunization programs [82,83]. Indeed, introducing news vaccines whose benefits may not be seen immediately constitute, with many other barriers, big challenges that faced most of Sub-Saharan African countries.

Difficulty in procuring vaccines due to high costs (approximately \$400 US for a three-series dose) is a major challenge. Fortunately, since nearly a decade, several initiatives are in place to reduce the cost of vaccination in Sub-Saharan African countries. One of the best examples is the Rwanda which was the first country to take advantage of the low pricing through a partnership with Merck, the pharmaceutical manufacturer of the Gardasil® vaccine and Qiagen (Qiagen, Gaithersburg, MD, USA). During 3 years, Rwanda therefore received a donation of two million doses of the quadrivalent vaccine Gardasil® from Merck and 250,000 HPV screening test from Qiagen. Now, the vaccine is offered at a high discounted price. With this partnership, Rwanda's HPV vaccination program achieved 93% coverage among girls aged 12–15 years in grade six [84].

As in Rwanda, the challenge posed by financial barriers is being overcome in other Sub-Saharan African countries by partnership between those countries and international financial support. GAVI then announced in May 2013, for the first time ever, a public offer for a price of \$4.50 per dose for both Gardasil® and Cervarix® to low-income countries, a drastic reduction from \$360 for the required three doses [85]. Kenya was then the first country to benefit from the financial support provided by GAVI to roll out a HPV vaccine pilot project. In 2014, GAVI also announced that they support the first nationwide introduction of HPV vaccine in Rwanda for girls of all eligible ages, as well as other HPV demonstration projects in

Mozambique, Zimbabwe, Ghana, Madagascar, Malawi, Niger, Sierra Leone, and Tanzania (Table 3) [86].

Vaccine delivery strategy (particularly in pubescent/adolescent population), low cervical cancer screening levels, poor health system capabilities, inaccessibility to medical care, low awareness and knowledge of HPV vaccination and cervical cancer are also many other barriers which are currently evaluated in Sub-Saharan African setting to implement and sustain effective vaccination programs (Figure 4(b)) [86]. The WHO Expanded Program of Immunization (EPI) has been successful in improving access and delivery of vaccines to children and achieving high coverage worldwide and particularly in Sub-Saharan African countries. However, even if HPV vaccines are incorporated into the EPI programs, there still remains the challenge of delivering a preadolescent/adolescent vaccine since no program exists targeting this population. To overcome this obstacle, several studies have been done, and others are ongoing [87,88].

Indeed, using a school-based pilot program supported by PATH International in 2009, Uganda's HPV vaccination program achieved 88.9% coverage among schoolgirls showing that HPV vaccine delivery at schools using grade eligibility was more feasible than selecting girls by age [86,89–91].

Pooled experiences afforded from 29 HPV vaccination programs implemented in 19 low- and middle-income countries during 2009–2014 have been reported, demonstrating that local organizations and institutions can implement successful HPV vaccination campaigns [92]. Moreover, adequate and adapted planning and resources that support information sharing, sensitization, and mobilization are essential for such success [92]. Recent studies in Sub-Saharan Africa on the safety and immunogenicity of HPV vaccines reveal that they are well-tolerated, safe, and immunogenic in girls and even in at-risk populations such as HIV-infected women [93–95]. Moreover, longtime after vaccination, one and two doses of the HPV-16/-18 vaccine seem to protect against cervical HPV-16/-18 infections, showing similar protection to that provided by three-dose schedule. These data argue for a direct assessment of one-dose efficacy of the HPV-16/-18 vaccine [96]. With the new promising microneedle patches technology, one-dose-based HPV vaccination programs could open up new perspective in vaccination in Sub-Saharan Africa, since a single-dose vaccine delivered by microneedle patches would make vaccination programs easier and much cheaper for African countries [97].

These implementation projects demonstrated that HPV vaccination is practical and safe in Sub-Saharan African school strategy. Political and community acceptance was good, and positive attitudes toward vaccination were encountered. Lessons learned in Uganda could be relevant for countries considering implementing HPV vaccinations.

More recently, a nonavalent HPV vaccine namely Gardasil® 9 has been developed [98]. This HPV (-6/-11/-16/-18/-31/-33/-45/-52/-58) virus-like particle vaccine targets five additional HR-HPV types compared with the Gardasil® 4 vaccine (Figure 4(a)). This broader spectrum vaccine could potentially increase HPV vaccination-related cervical cancer prevention to 90% [98]. Although there are very little data about this new vaccine in the Sub-Saharan African context, this is a promising

Table 3. African countries where Gardasil® is approved (including GAVI-eligible African countries).

African countries where Gardasil® is approved	GAVI-eligible African countries for funding for a Gardasil®-based vaccination program in 2016
Botswana, Congo, Egypt, Equatorial Guinea, Gabon, Mauritius, Morocco, Namibia, South Africa, and Tunisia	Benin, Burkina Faso, Burundi, Cameroon, Central African Republic, Chad, Côte d'Ivoire, Democratic Republic of Congo, Djibouti, Eritrea, Ethiopia, Gambia, Ghana, Guinea Conakry, Guinea Bissau, Kenya, Lesotho, Liberia, Madagascar, Malawi, Mali, Mauritania, Mozambique, Niger, Nigeria, Rwanda, São Tomé e Príncipe, Senegal, Sierra Leone, Somalia, Republic of Sudan, Tanzania, Togo, Uganda, Zambia, and Zimbabwe

Source: GAVI, 2015.

innovation for this area, which harbors a broad heterogeneity of HPV genotypes and where the predominance of HPV-16 and HPV-18 is not always respected [36]. The evaluation of this vaccine is still ongoing worldwide. Recently, a mathematical model analysis was performed by Kiatpongsan et al. [99] to determine a range of vaccine costs for which the 9valent vaccine would be cost-effective in comparison to the current vaccines in Kenya and Uganda, two less developed Sub-Saharan African countries [99]. The estimated prices provided by this simulation represent a 71% and 61% increase over the price offered to the GAVI Alliance (\$5 per dose) for the currently available bi- and quadrivalent vaccines in Kenya and Uganda, respectively. Therefore, this study provides evidence about the cost-effectiveness of the nonavalent HPV vaccine-based future program in Sub-Saharan African context.

8. International partnership and funding to fight HPV-related cancer in Africa

The prevention and management of HPV infections in Africa take on a regional dimension. However, to date, no country in Sub-Saharan Africa has been able to cope with the high and unavoidable costs of fighting the public health problem of cervical cancer at all levels. The urgency of the situation has forced some countries to initiate national programs to fight against cervical cancer, which must be sustained in order to be effective. Subregional pilot programs are implemented, as in Francophone Africa (COFAC-col program, Cameroon, Côte d'Ivoire, Gabon, Madagascar, and Senegal) [100] or in Southern and Central Africa [101], possibly by non-governmental organizations such as pink ribbon/red ribbon (Botswana, Ethiopia, Namibia, Tanzania, Uganda, the Democratic Republic of the Congo, and Zambia) [102]. Similarly, strategies specific to the Sub-Saharan context based on effective, rapid, low-cost, and mobile screening tools, but also vaccination and awareness rising, are evaluated on the ground. Significant progress has been made, suggesting the feasibility of these control programs across the continent [55]. However, many challenges remain. The collaboration of the various actors is insufficient, and the isolated initiatives lack coordination. All stakeholders, including national public health policies, non governmental organizations (GAVI and PATH) and international institutions (WHO, the United Nations, the United Nations Children's Fund, International Monetary Fund, World Bank, and African Development Bank), health professionals, scientists, governments of African states and private partners (pharmaceutical companies, vaccine manufacturers and test-takers) must work together in order to overcome these obstacles and effectively combat the scourge of cervical cancer [103].

9. Conclusion

Actually, cervical cancer remains a major public health problem in Sub-Saharan Africa. Forecasts for the future years are also very alarming. Indeed, the steady increase in maternal mortality associated to this cancer tends to annihilate more than 25 years of efforts undertaken by national and international public health policies to achieve one of the Millennium

Development Goals: the reduction of maternal mortality in the world. The main reasons for this worrying situation are the high prevalence of HPV infection in African populations, the persistent HIV epidemic, the ineffectiveness of current prevention programs, growing demographics, and the increase in life expectancy. Innovative prevention strategies based on effective, rapid, inexpensive, and mobile screening tools, including at best molecular biology as well as vaccination and sensitization programs, should be rapidly implemented and evaluated in Sub-Saharan Africa. MDGs deadline expired; to reach the new UN SDGs, several efforts must be done by national as well as international public health policies to sustain the implementation and the effectiveness of these prevention strategies in African settings.

10. Expert commentary

In the field of biological diagnosis, recent advances in molecular biology techniques make it possible to carry out a very early diagnosis of persistent genital in HR-HPV infection in women up to 30 years of age (testing below 30 years is not recommended because most early infections are transient), and thus to envisage a close monitoring of the patients, long before the development of precancerous lesions or even invasive (Table 2). Since cervical smears are a good screening tool for HIV-positive women, there is no need for systematic colposcopy [104]. The combination of cytology and HPV testing has been shown to marginally reduce the risk of cervical cancer and that only in a wealthy country like USA not Africa. Recently, the World Health Organization has updated his guide to prevent and control cervical cancer. The 'Comprehensive cervical cancer control: a guide to essential practice' also known as 'Pink Book' aims to help countries better prevent and control cervical cancer. The new guidance identifies key opportunities and ages throughout a woman's life when cervical control and prevention can be put into action, especially for HPV vaccination as primary prevention which targets girls aged 9–13 years, aiming to reach them before they become sexually active, secondary prevention with access to screening technology for women over 30 years of age, such as VIA or HPV testing for screening, followed by treatment of detected precancerous lesions, which may develop into cervical cancer and the access to cancer treatment and management for women of any age, including surgery, chemotherapy, and radiotherapy as tertiary prevention and palliative care when curative treatment is no longer an option. The guidelines also show how cervical prevention and control can be integrated into existing health care delivery systems, including for family planning, postpartum care, and HIV/AIDS. The guidance underlines the importance of collaboration between sectors, between health programs, and between professionals working at all levels of the health service, for the success of cervical cancer prevention [105].

The schematic implementation of public health interventions in Africa for the prevention, diagnosis, and management of HPV infections and cervical cancer lesions follows a programmatic progression at the regional and national levels (Figure 6(a)). In the area of care, diagnostic tools and curative tools, as well as dedicated medical and sociocultural

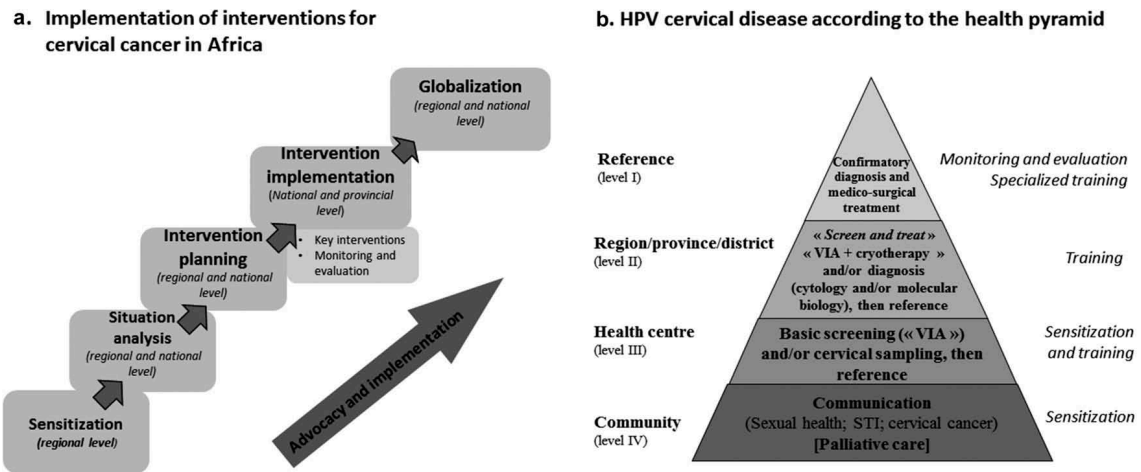


Figure 6. Strategies and intervention for cervical cancer prevention in Africa. a. Implementation at regional and national levels of public health interventions in Africa for the prevention, the diagnosis and the care of HPV infections and cervical cancer, according to the facilities of the health pyramid. 'VIA': Visual inspection with acetic acid; STI: Sexually transmitted infection

personnel, follow nationally the organization of the health pyramid (Figure 6(b)) [75]. At the community and voluntary level, the emphasis is on sensitizing women to sexual health, different STIs, and the risk of cervical cancer. Palliative care may possibly be organized at community level, as well as monitoring and socio-community integration of the cases treated. At the health center level, a simple visual screening can be proposed, or even a cervical smear or specimen sent to a higher level for diagnostic purposes; it is not possible to treat a possible lesion. At the provincial hospital level, treatment can be carried out according to the 'screen and treat' strategy, or a cervical smear or a genital specimen for diagnostic molecular biology for follow-up or treatment charge. Finally, at the higher level, the cytological and pathological diagnosis, HPV molecular detection and typing, and the medico-surgical management are carried out in a specialized multidisciplinary reference center where the staff training as well as specialized training of medical personnel, monitoring and evaluation of the prevention and treatment program of cervical cancer are also carried out.

11. Five-year view

The rate of cervical cancer-related mortality in Sub-Saharan Africa could be dramatically reduced at the same levels as those of developed countries by improving prevention strategies like optimizing the vaccine coverage, the reinforcement, and the sustainability of screening programs and treatment of cervical cancer. These strategies, which have been successful in developed countries, are found to be inadequate for developing countries, requiring a specific adaptation to Sub-Saharan African context. According to the results of studies conducted over the past 6 years, many Sub-Saharan African countries in collaboration have done several promising efforts with private sector partners, vaccine manufacturers, and international financial supports. However, most of these programs when they exist are only in their implementation stage and despite giving good preliminary results, several challenges

remain to be overcome in order to sustain. Thus, cervical cancer is a late-onset disease with an incubation period of up to about 30 years. Moreover, vaccinating pubescent/adolescent girls will significantly influence the incidence of this cancer only when they will reach their adulthood. So, the impact of recent projects initiated for less than a decade ago in Sub-Saharan Africa will not be truly appreciated in the next 5 years. According to GLOBOCAN 2012, the forecasts for the coming 5 years on the burden of cervical cancer in Sub-Saharan Africa are not favorable if the current state of prevention of this cancer remains the same.

However, if things change and move we have great hopes notably by further supporting vaccine-based prevention strategies as seen in the 10 years of real-world experience review done by Suzanne Garland about the fast decrease in most of HR-HPV infection rates, genital warts, and high-grade histologically proven cervical cancerous lesions [106]. Based on the lessons learned on the results of cervical cancer prevention since the recent past decades, UN General assembly on non-communicable disease has clearly established HPV and cervical cancer prevention in developing countries as funding priority [107]. Likewise, MDGs deadline expired; in order to continue and reach goals previously set in MDGs, the new SDGs have also made HPV and cervical cancer prevention a priority [108].

Key issues

- The high burden of cervical cancer in sub-Saharan Africa is a real health problem but this non-communicable disease remains until recently under-prioritized in most of sub-Saharan African countries. Indeed, the sub-Saharan African countries mainly support almost 85% of the global burden of cervical cancer. Moreover, the trends for the coming decades are not favorable, with nearly 90% of the global burden of cervical cancer, which will be supported by sub-Saharan African women in 2030.

- As it is commonly described around the world, HPV-16 and HPV-18 are the most prevalent genotype in sub-Saharan African population. However, some data show relative heterogeneity in the distribution of the most predominant HPV genotypes in certain region in sub-Saharan Africa supporting the critical need of the available new generation broad spectrum Gardasil® 9valent HPV vaccine.
- The HIV/AIDS epidemic is one of the major factors aggravating the burden of HPV-related cervical cancer in sub-Saharan Africa. Indeed, HIV-induced immune depression increased the risk for acquiring single or multiple high risk-HPV infections in sub-Saharan African women. It remains important to closely screen and monitor HPV infection and cervical abnormalities in HIV-infected women, particularly in the era of intensive widespread use of combination antiretroviral treatment in sub-Saharan Africa and its consequences on the life expectancy of the HIV-infected women.
- One of the main reasons that could explain the steady increased of the high burden of cervical cancer in sub-Saharan Africa is the failure state of cervical screening, the lack of robust and effective national prevention programs and medical management. Strong partnerships between national health policies, health international organizations, international financial supports and private partners such as screening test manufacturers could facilitate the integration and the widespread use of the promising alternative approaches to cytology such as visual inspection of the cervix and HPV DNA testing.
- Introducing news vaccines whose benefits may not be seen immediately such as HPV vaccines constitute a big challenge that faced most of sub-Saharan African countries. Moreover, many other barriers such as high private market costs of HPV vaccines, vaccine delivery strategy and the target population (pubescent/adolescent before first sexual intercourses), low awareness and knowledge of HPV vaccination, the inaccessibility to medical care are some of the other obstacles which must be overcome to implement strong and efficacious primary prevention against HPV infection-associated cervical cancer in sub-Saharan Africa. Fortunately, several initiatives and pilot projects like GAVI are in place to lighten the cost of vaccination in sub-Saharan African countries, drastically reducing its price until making it almost free for the populations. Likewise, promising results of evaluation projects for implementing HPV vaccines demonstrated that HPV vaccination is practical and safe in sub-Saharan African schools-based strategy.

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III. Immunité humorale naturelle et vaccino-induite dans les compartiments systémiques et muqueux au cours de l'infection à Papillomavirus humains

Avant-propos: Cette partie est une synthèse de la littérature décrivant les principaux travaux réalisés et les principales découvertes associées à la compréhension de l'immunité humorale associée à l'infection à HPV, mais également l'immunité humorale induite à la suite de la vaccination par les vaccins prophylactique anti-HPV. Cette revue de la littérature intitulée : **“Natural and vaccine-induced B cell-derived systemic and mucosal humoral immunity to human papillomavirus”** a été soumise dans le journal *“Expert review of anti-infective chemotherapy”*.

Résumé:

Introduction: On sait que les infections à Papillomavirus humain (HPV) jouent un rôle important dans la pathogenèse des néoplasies cervicales. Compte tenu de la morbidité et de la mortalité associées au cancer du col utérin, aux cancers de l'anus et de l'oropharynx, l'infection à HPV peut être considérée comme un problème de santé publique mondial, en particulier dans les pays en voie de développement. Les défenses immunitaires de l'hôte, impliquant une réponse immunitaire adaptative spécifique du HPV avec des cellules T CD8+ cytotoxiques ainsi qu'une réponse humorale dérivée des cellules B, contrôlent la plupart des infections à HPV avant le développement du cancer.

Domaines couverts: Dans cet article, nous synthétisons les connaissances actuelles sur l'immunité humorale systémique et muqueuse contre les HPV, au cours de l'évolution naturelle de l'infection et après la vaccination prophylactique, afin d'aborder les problèmes d'équilibre entre élimination virale et échappement au système immunitaire de l'hôte par le virus, la différence de réactivité croisée et d'avidité des anticorps anti-HPV naturellement induits et ceux induits par la vaccination, la relation fonctionnelle entre les classes d'anticorps spécifiques des HPV et leur pouvoir neutralisant, la possibilité de corrélats potentiels de protection naturelle et de substituts de protection par le biais de l'immunité humorale vaccino-induite, ainsi que la nature de la réponse immunitaire humorale induite par un les vaccins prophylactiques, capable de renforcer ou de compléter la réponse immunitaire humorale naturelle contre les HPV.

Opinion d'expert: Comprendre le rôle de l'immunité humorale spécifique contre les HPV au cours d'une infection naturelle est crucial pour la conception et l'amélioration des vaccins prophylactiques contre les HPV.

Natural and vaccine-induced B cell-derived systemic and mucosal humoral immunity to human papillomavirus

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Running head: Natural and vaccine-induced humoral immunity to HPV

Keywords: Human papillomavirus; Humoral immunity; B-cell; Prophylactic HPV vaccine; Virus-like particles; IgG; IgA; Immunogenicity; Mucosal humoral immunity; Systemic humoral immunity; cross-reactivity; neutralization

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Abstract

Introduction: Human papillomavirus (HPV) infections are known to play an important role in the pathogenesis of cervical neoplasia. Considering the morbidity and mortality of cervical cancer, anal and oropharyngeal cancers, infection with HPV can be regarded as a worldwide problem, especially in developing countries. Host immune defenses involving HPV-specific adaptive immune response with CD8⁺ T cells cytotoxic as well as B cells-derived humoral responses control most HPV infections before the development of cancers.

Areas covered: In this review, we synthesize current knowledge on systemic and mucosal humoral immunity against HPV, during natural history of infection and after prophylactic vaccination, in order to address the issues of the balance between viral elimination and viral escape to the host humoral immune response, the differential cross-reactivity and antibody avidity of natural and vaccine-induced HPV-specific antibodies, the functional relationship between HPV-specific antibody classes and their neutralizing power, the possibility of potential protection correlates and surrogates of protection through natural and vaccine-induced immunity as well as the nature of the HPV type-specific vaccine-elicited humoral immune response able to strengthen or supplement the natural HPV humoral immune response.

Expert opinion: Understanding the role of HPV-specific humoral immunity during natural HPV infection is crucial for the design and improvement of prophylactic HPV vaccines.

1. Introduction

Human papillomavirus (HPV) are a large family of 8,000 base-pairs, nonenveloped and double-stranded DNA epitheliotropic viruses that are the causative agents of anogenital and skin warts and also oral, anal and cervical low- and high- grade intraepithelial lesions and cancers (Doorbar et al., 2015; Mboumba Bouassa et al., 2017). More than 200 genotypes have been isolated and characterized, and more than one-fifth of them can be easily spread through direct sexual contact or through skin-to-skin contact from infected individuals to healthy people (Doorbar et al., 2015; Mboumba Bouassa et al., 2017). With an overall incidence of more than 30 million new cases per year, HPV infection is probably the most common viral sexually transmitted infection (STI) in the world (Doorbar et al., 2015; Mboumba Bouassa et al., 2017). The Center for Disease Control and Prevention (CDC), Atlanta, USA, estimates that more than 90% and 80%, respectively, of sexually active men and women will be infected with at least one type of HPV at some point in their lives (Chesson et al., 2014; Mboumba Bouassa et al., 2017).

More than 100 distinct HPV types isolated from humans have been completely described (de Villiers et al., 2004). Based on the nucleotide sequence encoding the major capsid protein L1, HPV systematics defines 16 genera (sharing less than 60% sequence identity) which encompass 44 species (sharing 60–70% sequence identity). Cutaneous HPV are found among the five genera α (species 2, 4 and 8), β , γ , μ , and ν , whereas the 48 HPV types infecting the mucosa belong exclusively to the genus α . The infection with certain mucosal HPV types leads to malignant cell proliferation (zur Hausen 1996). At least nineteen HPV of genus α are involved in HPV-associated cancers, including thirteen high-risk (HR)-HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), recognized as carcinogenic (IARC 2007), and 6 genotypes categorized as probably carcinogenic (HPV-26, 61, 66, 69, 73 and 82) (Munoz et al., 2003; Bouvard et al., 2009). HPV-16 and HPV-18 cause about 70% of cervical cancers in women worldwide and 90% of anal cancer in men, especially men who have sex with men (MSM) (de Sanjose et al., 2010; Elizabeth et al., 2016).

Sexually transmitted HPV types fall into two categories according to their implication in benign warts or invasive anogenital cancers (Bouvard et al., 2009). Low risk (LR)-HPV do not cause cancer but induce epithelial warts (or condylomata acuminata) in anogenital mucosa or around, and eight of them (HPV-6, 11, 40, 42, 43, 53, 54 and 70) are the most detected genotypes with HPV types 6 and 11 accounting for nearly 90% of all genital warts worldwide (Bouvard et al., 2009). LR-HPV are also associated with recurrent respiratory papillomatosis, a less common HPV-related disease characterized by benign tumors of the respiratory system (Bouvard et al., 2009).

Most HR-HPV infections occur early in the young age, at sexual onset, without any symptoms and may be cleared within 1 to 2 years by the host immune system (Doorbar et al., 2015). It is generally admitted that 80% to 90% of HPV infections, including asymptomatic infections, genital warts, or even high-grade genital lesions, are eliminated (Stanley 2008; Doorbar et al., 2015), indicating that the host immune defenses control most HPV infections before the development of cancers (Doorbar et al., 2015). During primary infection, the uptake of the virus into epithelial cells is thought to induce a pro-inflammatory microenvironment activating the adaptive immune system via the local antigen-presenting cells (APCs) (Roden & Stern, 2018). Thereafter, the activated APCs migrate to the local or regional lymph nodes and stimulate viral-antigen-specific CD4⁺ T cells which participate to the HPV-specific adaptive immune response in activating CD8⁺ T cells cytotoxic as well as B cells-derived humoral responses. It is suggested that HPV antigen-specific T cell-mediated immune response would constitute the effector arm participating largely to the elimination of HPV virions and HPV-related lesions (Bourgault et al., 2004; Stanley 2008; Stanley et al., 2014; Mollers et al., 2013). Indeed, the HPV-specific CD8⁺ T cell-mediated immune response allows to specifically target and destroyed HPV-infected cells. In addition, B cells-derived neutralizing antibodies able to neutralize circulating free HPV particles will act to further prevent subsequent HPV infections. Together, these two arms of the immune response help to control and clear HPV infection during the natural history of HPV infection (Roden & Stern, 2018). However, some HPV infections can escape the host immune defenses and persist latently for many years. Afterward, persistent infections with HR-HPV types can induce cell changes leading to low- and high-grade intraepithelial lesions, which may progress to cancer without adequate treatment (Doorbar et al., 2015).

Cervical, anal and oropharyngeal cancers associated to persistent HPV infections are raised in several regions around the world (Elizabeth et al., 2016; Mboumba Bouassa et al., 2018). One of the main strategies against these cancers is obviously the primary prevention against HPV infection by the HPV vaccine immunization of young girls and boys early, between 9 to 14 years, preferentially before sexual onset and any genital contact with HPV (Elizabeth et al., 2016; Mboumba Bouassa et al., 2017). However, for the key population of MSM, which is at high risk of HPV infection and related cancers, and for whom the age of sexual onset is particularly variable, HPV-vaccine policies in several settings recommend an extension of immunization up to 26 years, irrespective of primary HPV DNA molecular detection (Sauvageau & Dufour-Turbis 2016). The three current prophylactic virus-like particles (VLP) HPV vaccines, including the bivalent Cervarix® containing VLPs from HPV-16 and HPV-18 (GlaxoSmithKline, Brentford, UK), the quadrivalent Gardasil-4® (VLPs

from HPV-6, -11, -16 and -18) and the nonavalent Gardasil-9® (VLPs from HPV-6, -11, -16, -18, -31, -33, -45, -52 and -58) (Merck & Co. Inc., NJ, USA), have been designed to prevent HPV infections by inducing HPV-specific activation of the B cells humoral response leading to the production of high titers of both systemic and mucosal HPV vaccine type-specific neutralizing antibodies (Harper & DeMars 2017; Pinto et al., 2018). The current HPV prophylactic vaccines allow protecting uninfected individuals against HPV infection and to strongly minimize the risk of HPV-related cancer (Harper & DeMars 2017; Pinto et al., 2018).

Taken together, both HPV-specific adaptive and vaccine-induced B cell-derived humoral immunity are at the heart of natural or prophylactic defenses able to control HPV infections. Many fundamental and practical issues have recently emerged in the complex pathophysiology of HPV mucosal infections, particularly concerning the specific humoral immune defenses against HPV. Several major research questions remain addressed such as the role of humoral systemic or mucosal immunity in the defenses against HPV, the possible mechanisms involved in the protection associated with the HPV prophylactic vaccine which is able to act at the mucosal entry door of the virus although being administered in the systemic compartment, as well as the nature of the HPV type-specific vaccine-induced humoral immune response which could be able to strengthen or supplement the acquired HPV humoral immune response already in place following natural HPV infection.

Finally, the purpose of this review is to synthesize current knowledge on humoral immunity against HPV, during the natural history of infection and after prophylactic vaccination; and also, to address the many research questions that arise especially about the mechanisms of action of prophylactic vaccination.

2. Adaptive B cell-derived humoral immunity against HPV

2.1. Productive and latent HPV viral cycles influence humoral immune response to HPV

HPV antigens elicits an HPV-specific humoral immune response in both the systemic and mucosal compartments.

The host humoral immune response to HPV is closely linked to the exclusively intraepithelial viral cycles of HPV infection (Figure 1).

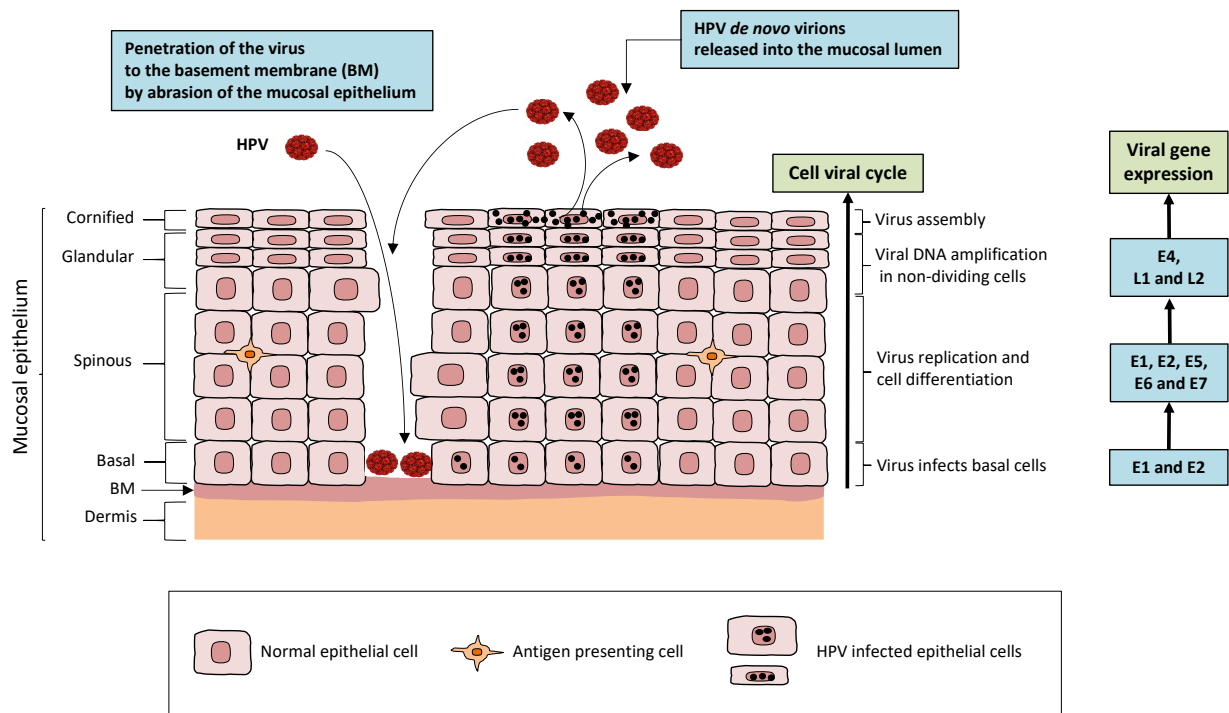


Figure 1. Natural history of HPV infection and viral cycle. *Human Papillomavirus (HPV) targeted cells are the epidermoid cells of the basal lamina of the mucosa. These cells are exposed to viruses only after deep lesions of the mucosa (e.g. during sex for the vaginal and anal mucosa). The viral particles in the lumen of the mucosa can thus reach the cells of the basal layer and infect them. The viral cycle of HPV is closely related to the natural desquamation of epithelial cells and involves different viral proteins depending on the stage of the viral cycle. Thus, in the early stages of infection that occur in the deep cell layer (basal, spinous cells) only the early non-constitutive viral proteins (E1, E2, E5, E6 and E7) involved in the replication of the viral DNA, are expressed, as the process of maturation and differentiation of epidermal cells occurs. The late genes (L1 and L2) coding for the structural proteins (L1 and L2) and the early E4 gene involved in the amplification of the viral DNA are expressed in the last stages of the viral cycle occurring in the differentiated mature cells. In the superficial outer layer of the mucosa, the HPV virions are then processed by encapsidating the viral DNA in the viral capsid. The release of virions in the lumen of the mucosa occurs with the desquamation of old cells, during the regeneration of the mucosa.*

The keratinocytes of the basal lamina of the mucosae exposed to HPV constitutes the main targets for viral particles, through surface micro-lesions in both anal and genital mucosae, frequently occurring during sexual intercourses (Doorbar et al., 2015; Roden & Stern 2018). Once the keratinocytes are infected, two viral processes can take place.

Firstly, a productive viral cycle may occur in the deepest cellular layers of the target mucosa characterized by the expression of the early genes (E1, E2, E4, E5, E6 and E7) of the

viral genome, which is closely dependent on the mucosal cell differentiation process. HPV virions are formed and mature in the cells of the superficial layers of the epithelial membrane to be released into the lumen of the mucosa during desquamation (Doorbar et al., 2015; Roden & Stern 2018). During viral replication, the late proteins (L1 and L2) are synthesized in the most differentiated and keratinized layers of the mucosa. The L1- and L2- genes-encoded capsid proteins constitute the main viral antigens which will be involved in the HPV-specific B cell immune response.

Secondly, the viral genome may be maintained in latency either in an episomal form within the nucleus or integrated in the host genome of the basal keratinocytes. During latent infection, only the early viral genes are expressed, while the genes coding for the late viral proteins are repressed. Thus, the nonproductive viral cycle is characterized by the presence of HPV DNA in the cells, without de novo production of HPV virion able to induce HPV-specific humoral immune response (Doorbar et al., 2015; Roden & Stern 2018).

The strictly intraepithelial viral cycles of HPV may be considered as a partial mechanism of viral escape to HPV-specific immune response. Thus, the first stages of the viral cycle constituted by the entry of the viral capsid into the keratinocytes should a priori provide an activation signal for the intraepithelial dendritic cells (DC) which are antigen presenting cells (APC) subpopulation of Langerhans cells (LC). However, using in vitro HPV infection model, it has been shown that LCs are not activated when they are incubated with HPV16 L1 derived virus-like particles (VLPs HPV-16 L1). The LCs did not initiate specific responses to VLPs, suggesting that in vivo, sentinel intraepithelial APCs, even in contact with real HPV particles, cannot be activated and therefore cannot initiate a specific and effective immune response (Fausch et al., 2002; Yang et al., 2005; Stanley 2008). In contrast, circulating stromal DCs in the genital tract may be activated by VLPs HPV16 L1 and stimulate differentiation of HPV-specific T CD4⁺ cells (Da Silva et al., 2007; Stanley 2008). However, since HPV remains almost exclusively buried in the epithelium, the likelihood of stromal DC being in contact with an HPV particle is very low (Stanley 2008). Thus, in the productive as well as the non-productive viral cycles, no inflammation or release of pro-inflammatory cytokines occur in the mucosal target epithelium, resulting in little or no danger signaling pathways that can activate the sentinel APCs (Roden & Stern 2018). Similarly, the release of viral particles which is closely related to the natural desquamation process of keratinocytes of the superficial membrane, is not exponential, but rather progressive, at the same rate as desquamation, producing only very few free viral particles at epithelial surface without viremia (Stanley 2008; Roden & Stern 2018).

In addition, although HPV are theoretically capable of binding to cells other than keratinocytes, the expression of viral genes and the production of viral proteins occur exclusively within keratinocytes, limiting the possibility of HPV viral proteins presentation to lymphocytes in peripheral lymphoid organs (Stanley 2008).

Taken together, HPV can escape the host immune system which appears not or only partially activated by viral proteins. However, the nonproductive HPV cycle that causes persistent infections induces, after a certain time, pathologic transformation of keratinocytes in the basal lamina causing dysplastic and metaplastic lesions of the mucosa that can progress to invasive cancer (Doorbar et al., 2015). In this latter situation, the early oncogenic proteins E6 and E7 interact with the cell cycle protein sensors (p53 and pRB) by inhibiting them and therefore are at the origin of the carcinogenesis process (Doorbar et al., 2015) with mucosal inflammation. The overproduction of the immunogenic E6 and E7 viral proteins further activates the host immune system (Doorbar et al., 2015).

2.2. Induction sites of acquired humoral immunity to HPV

HPV infection is a cutaneous and mucous infection. In case of strictly cutaneous HPV infection, specific immunity is part of the systemic immunity with antigenic presentation to APCs of the epithelial layer. In case of mucosal HPV infection, the mucosal associated lymphoid tissue (MALT) is involved.

A single contact with cutaneous HPV types might not result in antibody induction. However, accumulation of cutaneous HPV infections over lifetime due to an increased exposure via intense skin contacts or micro-abrasion of the squamous skin epithelium could lead to higher overall viral loads, resulting to an increasing seroprevalence with age (Michael et al., 2008).

For both HR-HPV (e.g. HPV -16, -18, -31, -33, -35, -45, -52, -58) and LR-HPV (e.g. HPV -6 and -11) mucosal HPV, transmission occurs mainly via sexual intercourse (Dillner, Andersson-Ellstrom et al., 1999). However, non-sexual routes of transmission, e.g. oral, anal and perinatal transmission have been reported and cannot be entirely disregarded (Syrjanen & Puranen 2000; Cason et al., 2005).

HPV infections of the penis involve rather keratinized than mucosal epithelium which might be less susceptible for the virus, less productive, and less accessible for the immune system. Men might have rather transient infections with lower viral loads that induce a weaker antibody response than in women (Michael et al., 2008).

For mucosal HPV infection, inductive sites are part of the mucosal immune system. The mucosal immune system is involved in the local defense against pathogens at mucosal sites and consists of sites where antigens are encountered and processed via the MALT (Brandtzaeg et al., 1989). MALT is present in the gastrointestinal and the respiratory tract as well as in the lower female genital tract. In the afferent phase, antigen is encountered at the mucosal surface by APCs and subsequently delivered to the underlying submucosal lymphoid tissue. This antigen uptake and presentation results in lymphocyte priming, which is followed by migration (“homing”) of T- and B-cells to mucosal effector sites via the regional draining lymph nodes and the peripheral blood (Brandtzaeg et al., 1989). Subsequently, precursor cells of cellular and humoral immunity, located in T- and B-cell zones, are stimulated, finally resulting in production of T helper cells, cytotoxic T cells and immunoglobulins at mucosal effector sites such as the lamina propria and the mucosal epithelium (Mestecky & Russell 1997). Secretory IgA (S-IgA) is the best-defined component of the immunoglobulins at mucosal surfaces (Brandtzaeg et al., 1995). In addition, IgG and secretory-IgM also contribute to mucosal surface protection (Brandtzaeg et al., 1991). Naive B cells require two distinct types of signals or stimuli for their proliferation and differentiation (Brandtzaeg et al., 1989). One type of signal is provided by the antigen interacting with membrane Ig molecules on specific B cells. The second type of signal is provided by Th2 associated cytokines secreted by helper T lymphocytes. Upon contact with antigen, specific B cells bind, internalize, and process the antigen and present peptide fragments of the antigen via MHC class II molecules to specific helper T cells.

Little is known of the afferent inductive phase of mucosal immunity in relation to HPV. For oral and anal HPV infections, inductive sites of HPV-specific mucosal immunity belong a priori to regionally specialized areas of the MALT, e.g. the oropharyngeal lymphoid tissues, including the Waldeyer’s ring and the tonsils, and the gastro-intestinal-associated lymphoid tissue (GALT), respectively.

For cervical HPV infection, the issue of inductive sites appears more complex (Figure 2). Thus, the female genital tract displays features that are different to other mucosal sites or the systemic compartment (Russell & Mestecky 2002). Biopsies taken from the vaginal and the ectocervical mucosa of the female lower genital tract revealed the presence of Langerhans dendritic cells and macrophages, which are important in the afferent phase of the immune response (Holmgren et al., 1991). In the submucosa, especially the transformation zone of the cervix, lymphocyte subsets and plasmocytes have been identified with a tendency to form lymphocyte aggregates (Kutteh et al., 1990; Brandtzaeg et al., 1997; Brandtzaeg et al., 1999). Thus, the lower female genital tract is an inductive site of cervicovaginal immunity (Kutteh &

Mestecky 1994). However, the cervix in particular has been characterized as being an immuno-privileged site with prevailing immuno-suppression, probably to allow for the safe passage of sperm and fetus (Hoglund et al., 2003). As a consequence, female genital inductive site is probably poorly involved during genital HPV infection, as long as no HPV-related cervical lesions has occurred (Hagensee et al., 2000). Little is known of the afferent genital inductive phase of mucosal immunity in relation to HPV, but a disturbance in the afferent phase of the immune response is suspected. For example, analysis of inflammatory infiltrates in cervical dysplasia has revealed reduced numbers of Langerhans' cells, cells that belong to the DC lineage, in CIN II and III lesions (McArdle et al., 1986; Tay et al., 1987; Hughes et al., 1988; Viac et al., 1990). Since DCs are generally accepted as being the most efficient APCs of the immune system (Hart et al., 1991), their lack could theoretically result in an inefficient primary immune response.

As part of the common mucosal immune system (Mestecky & Fultz 1999), other inductive sites from the MALT, especially those of the GALT and particularly the anal-associated lymphoid tissue, far from the genital mucosa itself, could be involved in the female genital humoral immune response to HPV.

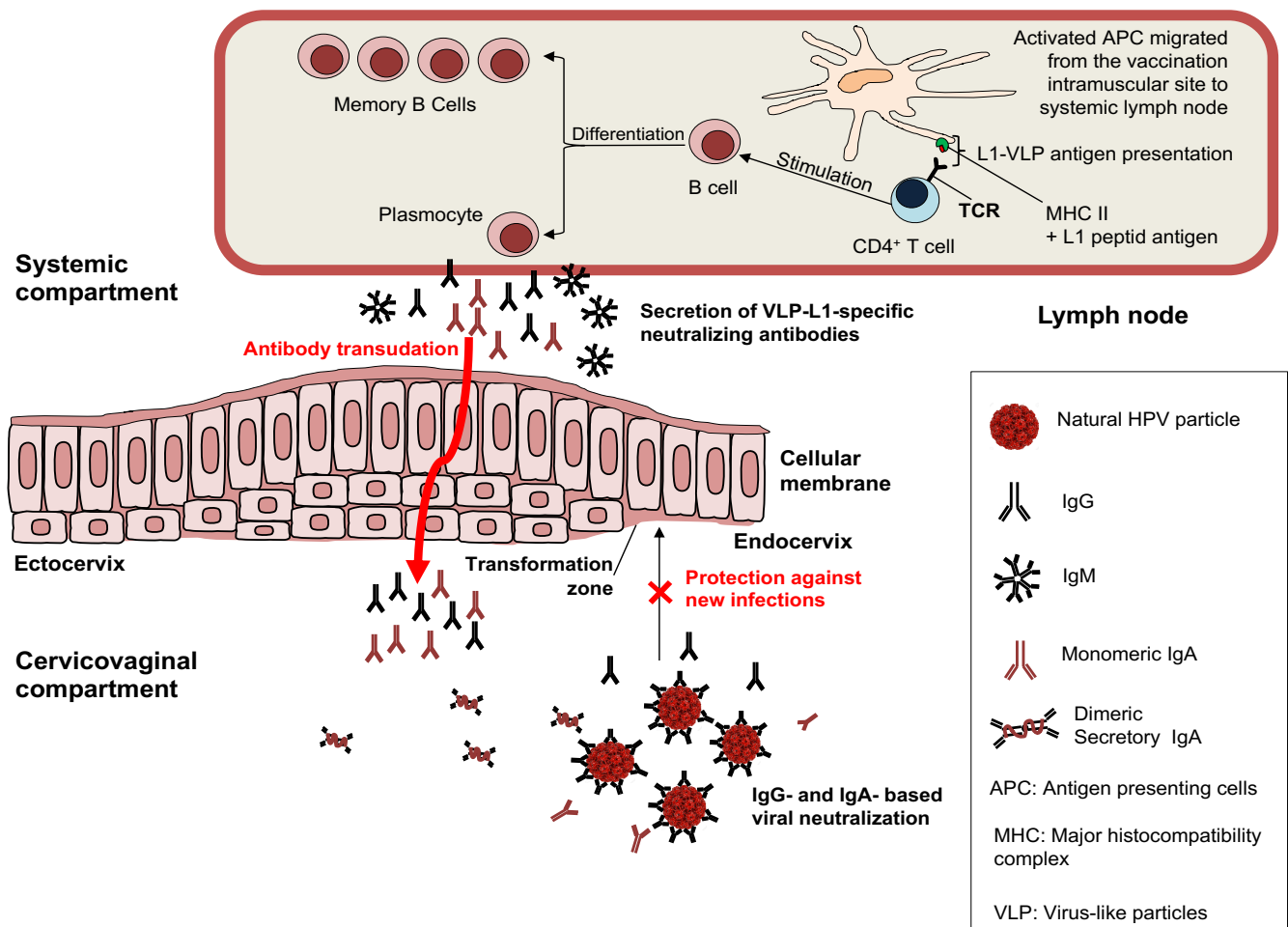


Figure 2. Humoral response mechanism induced by HPV vaccination. *After intramuscular inoculation of virus-like particles (VLPs) contained in the HPV vaccine dose, sentinel cells of natural immunity, such as dendritic cells, incorporate and proceed to the phagocytosis of vaccine VLPs. Dendritic cells, which are antigen presenting cells (APCs), express on their surface, conformational peptides derived from the phagocytosis of vaccine VLPs. APCs then migrate into peripheral lymph nodes around the vaccination site, where they stimulate the immune cells of adaptive immunity. In the case of a humoral adaptive response with ultimately the secretion of antibodies directed against the presented antigen, the APCs present the antigen to CD4⁺ T cells via the major histocompatibility complex (MHC) type II. Once the LT-CD4⁺ are activated, they stimulate the proliferation and differentiation of the B cells, specific to the VLP antigen presented. The selected B cells line will then differentiate into plasmocytes able to secrete antibodies specific for the antigen presented, but also in memory B lymphocytes. The plasmocytes will then secrete into the blood system, different isotypes of immunoglobulins (IgA, IgG and IgM), all specific to the antigen presented. In order to ensure the protection of the mucosa, the gateway of the HPV infection, circulating serum IgG and IgA isotypes will cross the membrane barrier separating the systemic compartment from the mucosal compartment, by transudation or exudation, to join the lumen of the mucosa. The APC surrounding the vaccination site could a priori also migrate towards lymph nodes associated with the genital compartment. In this case, activated local B cells could secrete, locally, both IgG, monomeric IgA, and secretory (mucosal-specific) IgA dimers directly into the mucus impregnating the walls of the genital mucosa. Together, these immunoglobulins specific to the conformational antigens of the L1 protein of the different types of VLP contained in the vaccine, will be able to recognize native viral particles present in the lumen of the mucosa, to fix and neutralize them before they infect epidermoid cells of the mucosa. This immunity acquired through intramuscular vaccination can thus ensure the protection of the vaginal mucosa, far from the site of vaccination. The memory B cells preserved in the lymph nodes allow this humoral immunity to be durable and put in place more quickly at the next intrusions of HPV.*

2.3. B-cell epitopes of HPV and conformational neutralizing antibodies

The HPV structural genes encode the major (L1) and minor (L2) proteins that form the nonenveloped icosahedral viral capsid, which comprises 72 pentameric L1 capsomers, and each capsomer has an upper estimate of one L2 protein (Buck et al., 2013; Wang et al., 2013). The L1 protein mediates attachment to host cells, while the L2 protein is essential for subsequent viral infectivity. The L1 protein can spontaneously self-assemble into virus-like particles (VLPs) (Kirnbauer et al., 1992), which are the basis of the current prophylactic HPV vaccines. In addition, the early (E1, E2, E4, E5, E6, E7) proteins interact with host and viral proteins to maintain viral replication. The late proteins form the viral protein coat during productive infections.

Several seroepidemiological studies of HPV infection have been undertaken over the last two decades. The humoral immune response following natural HPV infection predominately targets conformational epitopes on the surface-exposed loop regions of the L1 protein (Carter et al., 1996; Carter et al., 2006). Although L1 specific antibodies played an essential role in the immune response against HPV infection as they allow neutralizing the viral particles and theoretically protect against subsequent infection, they are not the only antigen specific antibodies elicited by the HPV-related diseases. Indeed, apart the L1 and L2 capsid proteins synthesized lately and make up the bulk of the virus, early proteins of HPV such as E6 and E7 are also produced in large quantities and therefore constitute powerful antigens (Kreimer et al., 2017; Lang Kuhs et al., 2017).

Natural infection with HPV or vaccination with HPV-L1-VLP-based vaccines can induce a complex antibody response (Zhang et al., 2016). A wide array of HPV-specific antibodies directed to different B-cell epitopes presented on the VLP surface can be elicited by the virions or VLP-based immunogens. The conformational B-cell neutralizing epitopes are localized on surface loops of the viral major capsid protein L1 of the virions, at various locations on the capsomere. The lack of permissive and productive cell cultures for HPV has however impeded the study of virus neutralizing mechanisms of antibodies. Nonetheless, the neutralizing antibodies are thought to usually exert their function by blocking the binding and entry of HPVs into host cells. Thus, the interaction between neutralizing antibodies and the major capsid protein can block the virions from binding to the target host cells, inhibiting the subsequent cell entry process, by inducing conformational changes in the viral capsid, which can result in concealing the binding site for the cellular receptor or in inducing premature genome release.

Finally, the population of antibodies produced in response to a particular antigenic stimulus such as HPV capsid antigens or a VLP, is heterogeneous. Most antigens are structurally complex, containing many different epitopes or antigenic determinants. The immune system responds to the antigen by producing antibodies to most of the accessible epitopes. Thus, in any response to a specific protein there will be several populations of antibodies; the overall antibody response is polyclonal or heterogeneous and it comprises the output of all the individuals' stimulated B cells. Epitopes recognized by B cells are usually a confirmation and these B cell epitopes are only displayed by proteins in the native or tertiary structure. Complex proteins such as L1 contain multiple overlapping epitopes, some of which are immunodominant, that is, they induce a more profound and stronger response in the host than other epitopes and therefore dominate the polyclonal response. The immunodominant antibodies are type-specific antibodies but there are, subpopulations of other antibodies, some

of which will be to epitopes shared by other HPV types. For example, HPV-16-specific antibody responses to several linear and conformational HPV epitopes are independently associated with cervical cancer (Dillner et al., 1995).

2.4. HPV genotype diversity and naturally acquired humoral immunity: mono-type and multi-type infections; cross-reactivity

With more than 200 identified HPV genotypes circulating in human population, multiple HPV infections of different genus are frequent in the same individual. Thus, multiple infections with mucosal HPV (Partridge & Koutsky 2006; Clifford et al., 2005) and even more often with cutaneous HPV both in immunosuppressed as well as immunocompetent individuals are common (Antonsson et al., 2000; Berkhout et al., 2000). Multiple seropositivity may result from type-specific reactions to multiple infections and/or could be due to cross-reactive antibodies induced by an infection with only one or few HPV types (Michael et al., 2008; Scherpenisse et al., 2013b).

HPV-specific serology provides a powerful epidemiological tool to investigate the wide variety of mucosal and cutaneous HPV types and their distribution in the population (Dillner et al., 1999; Scherpenisse et al., 2013b).

For mucosal HPV types of the genus α , antibodies to the viral major capsid protein L1 have been shown to be markers for present and past infection. To our knowledge, mucosal HPV L1 antibody analyses have been performed so far for HR-HPV types -16, -18, -31, -33, -39, -45, -52, -58, -59, -73, and for LR-HPV types -6 and -11. Serology specific to HPV-16 may be used as a proxy for lifetime cumulative exposure (Dillner et al., 1999). HPV-16 L1 antibodies have low but stable titres and can be detected decades after the infection (Dillner et al., 1999). It is not clear whether naturally derived HPV-specific antibodies can protect against subsequent homologous infection or against infection with phylogenetically related HPV genotypes (Ho et al., 2002; Palmroth et al., 2010). In unvaccinated, HPV-antibody-positive individuals, natural antibodies to HPV-16 L1 have been shown to be primarily type-specific (Dillner et al., 1999; Coursaget et al., 2003; Palmroth et al., 2010; Scherpenisse et al., 2013b), although some degree of cross-reactivity with closely related types was reported (Coursaget et al., 2003). Scherpenisse and colleagues found that naturally induced HPV-16 and -18 antibodies in single-positive sera were non-cross-reactive (Scherpenisse et al., 2013b). In addition, single-positive sera showed neutralizing capacity, while antibodies in multi-positive sera were cross-reactive and non-neutralizing, suggesting that in single-positive sera the HPV-specific antibodies might be induced by transient HPV infections, whereas for

multi-positive sera the HPV-specific antibodies might be induced after persistent HPV infections (Scherpenisse et al., 2013b). For the other HPV types of the genus α , similar characteristics of L1 antibodies are assumed.

Michael and colleagues drew an epidemiologic profile of HPV infection by 34 HPV types (29 cutaneous and five mucosal) from 15 species within five phylogenetic genera (α , β , γ , μ , ν) in the general German population (Michael et al., 2008). Antibodies reactive with cutaneous types were the most prevalent, with genus ν (HPV-41) and μ (HPV-1 and HPV-63) being the most detected. The antibody prevalence to mucosal high-risk types, most prominently HPV-16, was elevated after puberty in women but not in men and peaked between 25 and 34 years. Antibodies to β and γ genus were rare in children and increased homogeneously with age, with prevalence peaks at 40 and 60 years in women and 50 and 70 years in men. Antibodies to cutaneous α genus showed a heterogeneous age distribution. These serological observations suggest different seroprevalence patterns of phylogenetically related HPV: Antibodies to cutaneous μ and ν HPV appear late in childhood, those to mucosal α HR-HPV after puberty mainly in women, and seroprevalence for β and γ HPV peaked late in life in both genders (Michael et al., 2008).

Both commercial prophylactic vaccines have shown evidence of cross-protection, or protection against non-vaccine HPV types. The high antibody concentrations generated by the vaccines probably explain this phenomenon (Scherpenisse et al., 2013b). In natural infections, the antibody concentrations generated are low so that only the immunodominant species is detected, but in VLP-immunized individuals, antibody concentrations are high and the subpopulations of cross-reactive and cross-neutralizing can therefore be detected in serological assays (Stanley et al., 2009; Scherpenisse et al., 2013b). These subpopulations are present at antibody concentrations one to two logs lower than the dominant type-specific neutralizing antibodies (Smith et al., 2007). Not every individual will generate cross-neutralizing antibodies since immunodominance is complex (Figure 3). Interestingly, the cross-protection afforded by the current prophylactic vaccines is an unexpected additional benefit. Indeed, in contrast to natural HPV-specific immunity, vaccine-induced humoral immune response against the seven $\alpha 7/\alpha 9$ HR-HPV types targeted by the Gardasil-9® multivalent vaccine could be efficient against a wide spectrum of vaccine and non-vaccine oncogenic HR-HPV types, because of high neutralizing capabilities and potent cross-reactivities within the same $\alpha 7$ or $\alpha 9$ HR-HPV species conferred by vaccine-derived HPV-specific antibodies (Draper et al., 2011; Malagon et al., 2012; Wheeler et al., 2012; De Vincenzo et al., 2013; Scherpenisse et al., 2013; Barzon et al., 2014; Bisset et al., 2014).

The criterion used to designate serotypes is generally based upon a fold difference in antibody-mediated neutralization titers between viral types. For HPV there are no currently defined criteria with which to designate L1 serotypes. It is reasonably clear that HPV genotypes induce high-titer, type-specific neutralizing antibody responses which represent different serotypes (Giroglou et al., 2001; Combita et al., 2002; Bisset et al., 2014). However, for lineage variants, the relationship between L1 sequence and antigenicity is less clear (Cheng et al., 1995), although HPV-31 variants have been shown to belong to a single L1 serotype (Bisset et al., 2015).

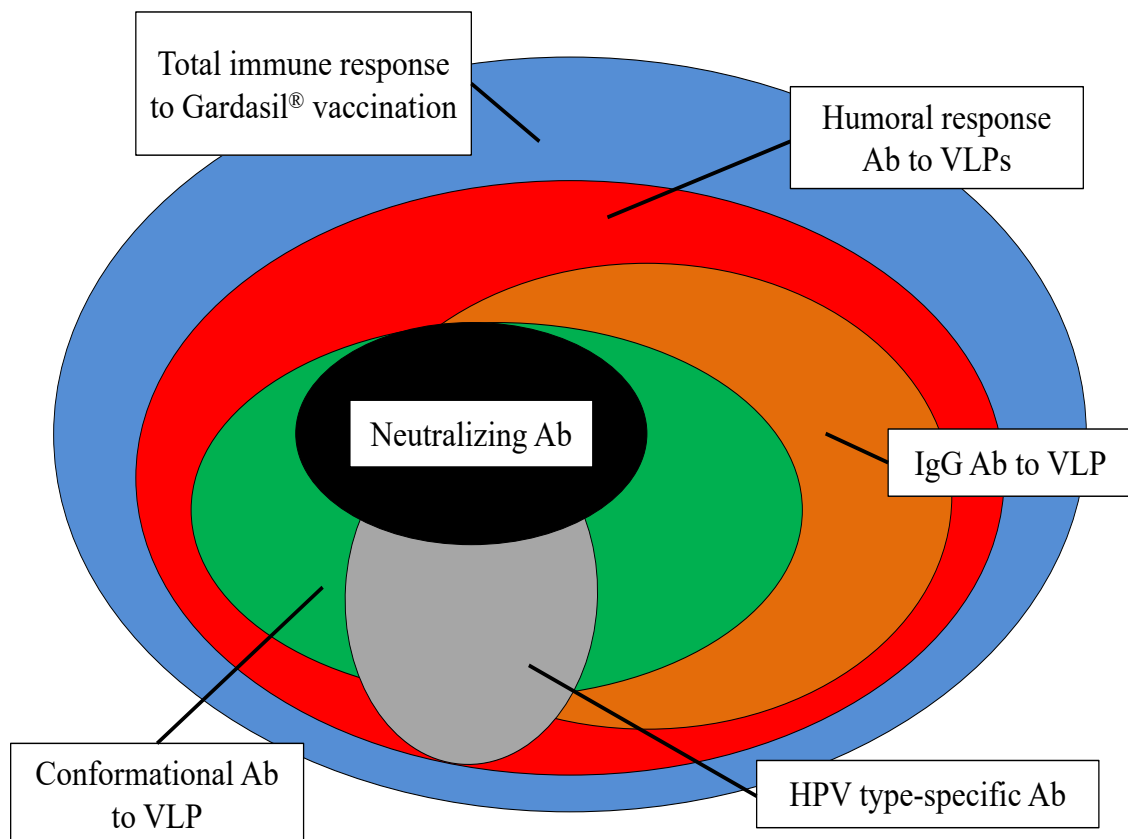


Figure 3. Schematic representation of the complex immune response to Gardasil® HPV prophylactic vaccine. *Overlapping circles indicate the combined attributes of antibodies within the circles. The global immune response induced by vaccination (blue zone) corresponds to the association between the cell-mediated immune response (cytotoxic response) whose effectors are CD8+ T cells and the humoral response (red zone) whose effectors are antibodies against the VLPs contained in the vaccine. This humoral immune response corresponds to a heterogeneous set of antibodies of various isotypes and idiotypes, all of which are induced by the VLPs contained in the vaccine (red zone). The most important antibodies in this set are the neutralizing antibodies (black zone). These antibodies correspond to the smallest fraction of all the antibodies induced by*

vaccination. These antibodies are specific for the HPV genotype corresponding to vaccine VLPs (gray area) and they recognize their target conformationally (green zone). These two characteristics allow these neutralizing antibodies to be able to inhibit HPV infection of the cells by neutralizing the native virus particles well before they enter the cells. These antibodies are the basis of the protective effect afforded by the vaccine humoral immune response. These antibodies consist mainly of IgG (brown zone), but also by other antibody isotypes (black zone located outside the brown zone). The neutralizing antibodies represent a fraction of the total immune response; that fraction being the potentially “protective” antibody population induced by Gardasil® vaccination. Adapted from (Smith et al., 2007).

3. Assays to evaluate humoral immune response to HPV

Despite the potential of HPV serology in disease diagnosis and prognosis, its clinical application has been limited by HPV heterogeneity, assay variability, and viral immune evasion. Serologic responses to HPV antigens are used as HPV exposure markers, stratification variables in epidemiological and physiopathological studies, and immunogenicity measurements serving as presumptive correlates of protection in vaccine clinical trials (Schiller et al., 2009; Pinto et al., 2018).

The main serological assays used to evaluate the humoral responses to HPV antigens acquired following natural HPV infection and after vaccination by the current HPV prophylactic vaccines are summarized in the Table 1. Briefly, three main HPV serological assays based on approaches that differ considerably in the potential relevance of detected antibodies are particularly used.

Table 1. Advantages and constraints of HPV serological assays (Pastrana et al., 2004; Dessy et al., 2008; Opalka et al., 2010; Robbins et al., 2014a; Pinto et al., 2018).

	Advantages	Constraints
L1&L2* PsV-based neutralization assay	<ul style="list-style-type: none"> Measures function closest to presumed mechanism of protection Detection of all immunoglobulin classes 	<ul style="list-style-type: none"> Requirement of one PsV for each genotype, which needs to incorporate a reporter gene Requirement of cell culture and time for cells to grow Time-consuming and labor intensive Limited ability to multiplex Higher coefficients of variation
Competitive Luminex™ immunoassay	<ul style="list-style-type: none"> Detection of neutralizing antibodies Robust and high throughput Easily multiplexed with bead arrays Rapid, high throughput Detection of all immunoglobulin classes 	<ul style="list-style-type: none"> Detection of only a subset of total neutralizing antibodies Use of type-specific neutralizing monoclonal antibodies Compromise between selecting dominant epitope and retaining type specificity because of multiplexing
ELISA		
L1 VLP-based ELISA	<ul style="list-style-type: none"> Fast, sensitive and high throughput Familiar assay format Amenable to multiplexing 	<ul style="list-style-type: none"> Detection of one immunoglobulin class (IgG or IgA), determined by secondary antibody Detection of non-neutralizing binding antibodies
Glutathione S-transferase L1 fusion protein assay	<ul style="list-style-type: none"> Fast and high throughput Familiar assay format Use of small sample amount Amenable to multiplexing Able to detect up to 100 different antigens simultaneously Scaled up for large studies with high sample size Low cost alternative 	<ul style="list-style-type: none"> Detection of one immunoglobulin class (IgG or IgA), determined by secondary antibody Detection of non-neutralizing binding antibodies

Firstly, the most relevant assay for measuring the biological activity of total antibodies is the in vitro HPV L1- and L2- based pseudovirus (PsV) neutralization assay (Pastrana et al., 2004). The HPV-PsV neutralization assay measures total immunoglobulins (IgM, IgA, and IgG) neutralization activity of serum or mucosal fluid on the infectivity of PsV in an in vitro cellular model (Pastrana et al., 2004; Buck et al., 2005; Sehr et al., 2013). The PsV-based neutralization assay was developed both to evaluate the functional assessment of the HPV neutralizing antibodies in sera elicited by vaccination or natural infection, to assess the structural basis for clinically relevant epitopes, and to determine the functional characteristics of monoclonal antibodies. In 2010, the World Health Organization (WHO) has proposed that the HPV-PsV neutralization assay should be considered as the gold-standard for assessing protective activity of the humoral immunity elicited by HPV prophylactic vaccines (WHO 2010).

The second most important assay is the competitive (epitope-specific) Luminex™ immunoassay (cLIA) (Opalka et al., 2010; Brown et al., 2011; Roberts et al., 2014). The cLIA estimate neutralizing activity of a human secretion (mostly serum) by evaluating competition

between a serum sample and labeled HPV epitope-specific monoclonal antibodies to bind in a specific epitope of HPV VLP (Roberts et al., 2014). The cLIA is highly specific. The assay may lack of sensitivity because it may underestimate the total functional antibody levels because the competition involves only certain epitopes (Roberts et al., 2014).

Another relevant tool for humoral immunity measurements is based on enzyme-linked immunosorbent assay (ELISA) using the L1 virus-like particles (VLP ELISA) (Dessy et al., 2008). VLP ELISA measures both neutralizing and non-neutralizing antibodies belonging to only one immunoglobulin class recognizing both conformational and non-conformational epitopes in L1 VLP previously fixed to a solid surface (beads or wells). Another ELISA-derived HPV serological test incorporating the so-called glutathione S-transferase technology has also been developed, which uses the glutathione S-transferase L1 fusion protein (GST-L1) (Robbins et al., 2014a; Robbins et al., 2014b). As for the VLP ELISA, the GST-L1 assay measures both neutralizing and non-neutralizing antibodies to HPV L1 protein and allows detecting up to 100 different antigens simultaneously (Waterboer et al., 2005).

The immunological assays for HPV-specific antibodies allow providing both qualitative (positive/negative) and quantitative (magnitude of antibody levels) insights on HPV-associated mucosal and systemic humoral immunity. These assays have been widely used to evaluate both the naturally acquired and vaccine-induced humoral immunity to HPV and data provided by these assays are generally well correlated (Dessy et al., 2008; Robbins et al., 2014a; Pinto et al., 2018). The interpretation of HPV serology is however complicated by substantial differences across assays used in different studies (e.g., detection ranges, targeted HPV types, and epitopes) (Schiffman et al., 2009; Safaeian et al., 2010; Lin et al., 2013). The standardization of immunological assays to HPV, in particular concerning the determination of positivity thresholds (cut-offs), as well as establishing the biological relevance of the results are currently become necessary in order to improve and facilitate data comparisons.

4. Detection rates of HPV-specific humoral immunity in natural HPV infection

In response to mucosal viral infection, the infected individuals may seroconvert and further secrete a large panel of serum and/or mucosal antibodies directed against viral antigens (Mestecky et al., 2005). This pool of antibodies consists mainly of IgG and IgA, both in the serum and the mucosal compartment at different levels (Mestecky et al., 2005).

Natural B cells-derived humoral responses to HPV has been largely described in the systemic compartment (Gissmann et al., 1996; Carter & Galloway 1997; Carter et al., 2000; Touzé et al., 2001; Stanley 2010; Scherpernisse et al., 2013). Most literature about HPV-

specific humoral immunity in natural HPV infection focused on serum HPV-specific antibodies, which reflects the humoral immune response to HPV in the systemic compartment (Stanley 2009; Stanley 2010).

The HPV-specific B-cells induced humoral immune response is weakly detected during natural infection in both women and men (Mollers et al., 2013; Beachler et al., 2016). As the infection is non-lytic, the host antibody response to L1 is weak and may persist for years, as an indication of past infection but not malignancy (Stanley 2010). Although anti-L1 antibodies are an indication of past infection, only 50 - 70% of infected women (Dillner et al., 1999; Carter et al., 2000) and 10 - 35% of infected men (Lu et al., 2012a) seroconvert (with wide range variability among series), demonstrating consistent gender gaps in population seroprevalences.

4.1. Adult female population

Only 50% to 70% of genital HPV DNA-positive women possess serum HPV-specific antibody, mainly directed against epitopes of the L1 capsid protein of the virus (Carter et al., 2000; Tong et al., 2013), suggesting that all women do not seroconvert after incident or even persistent HPV infections (Mollers et al., 2013; Velentzis et al., 2014; Beachler et al., 2016). Thus, the relationship between HPV DNA in the genital mucosa and serum IgG to HPV-16, -18, and -6 was studied prospectively in a cohort of 588 college women in order to address the kinetics of antibody responses in relationship to HPV DNA detection (Carter et al., 2000). At enrollment, the majority of women in the cohort had few or no sex partners; therefore, the HPVs detected were likely to represent their first exposure to the virus, rather than reactivation of latent infections. For all 3 types of HPV, the kinetics and magnitude of antibody responses following the detection of HPV DNA varied considerably from 1 individual to another. Some women generated vigorous sustained antibody responses, whereas others had no detectable reactivity. Among women with incident HPV infections, 59.5%, 54.1%, and 68.8% seroconverted for HPV-16, -18, or -6, respectively, within 18 months of detecting the corresponding HPV DNA. The median time to seroconversion ranged from 11.7 months for HPV-6 to 12.6 months for HPV-18. HPV-16 was found to have a median time to seroconversion of 11.8 months. Detection of serum HPV-16 antibodies was generally associated with HPV-16 DNA persistence, as previously reported (Wideroff et al., 1995; Carter et al., 1996; de Gruijl et al., 1997; de Gruijl et al., 1999). However, some women with persistent HPV DNA never seroconverted (Carter et al., 2000). Among women with the most vigorous antibody responses, those with HPV-16 produced stronger responses than those

with HPV-6 or -18. In contrast, transient HPV DNA was generally associated with a failure to seroconvert following incident HPV infection. Previous studies had yet demonstrated that a significant proportion of women with HPV infections do not develop detectable serum antibodies (Kirnbauer et al., 1994; de Gruijl et al., 1997), and others have shown that not all women develop mucosal antibodies (Bontkes et al., 1999). Antibody responses to each type were heterogeneous, but several type-specific differences were found: seroconversion for HPV-16 occurred most frequently between 6 and 12 months of DNA detection, but seroconversion for HPV-6 coincided with DNA detection (Carter et al., 2000). Additionally, antibody responses to HPV-16 and -18 were significantly more likely to persist during follow-up than antibodies to HPV-6, indicating type-specific differences in antibody persistence (Carter et al., 2000). These findings are consistent both with studies of HPV-16 antibody persistence that have concluded that serum antibody responses persist for years (Af Geijersstam et al., 1998; Shah et al., 1997) and with studies indicating that HPV-6 antibodies are often lost after treatment (Wikstrom et al., 1995; Elfren et al., 1996). In summary, the proportion of women who seroconverted was ~60% within 18 months of DNA detection for all 3 types. Furthermore, HPV-16 and -18 antibody responses were more likely than HPV-6 antibody responses to persist throughout follow-up.

Low rates of HPV-specific detection have also been reported at mucosal sites. Thus, the rates of IgG and IgA to VLP-16 in cervicovaginal secretions were detected infrequently (between 10-14%) in female sex workers at high risk for HPV-16 living in South Africa (Marais et al., 2009).

4.2. Adult male population

While most (50%-70%) unvaccinated women with natural incident or persistent genital HPV infection develop a measurable HPV-specific antibody response both in genital mucosa and serum, several studies have shown that lower rates of HPV-infected men exhibited HPV-specific humoral response, depending on several factors including the age, the sexual orientation and practices and also the anatomic sites (Lu et al., 2012a; Lu et al., 2012b; Giuliano et al., 2015; Beachler et al., 2016; Pamnani et al., 2016). For example, in men residing in Brazil, the prevalences of HPV-16 VLP -specific antibodies was 12.3% overall, 10.0% among men who have sex with women (MSW), and 31.2% among MSM (Lu et al., 2012a). Overall, seronegative and seropositive men differed significantly by age at enrollment, country of residence, the number of lifetime sex partners (either sex), and new sex partners (either sex) in the past 6 months. Seropositive men were more likely to be 25 years or

older, Brazilian, and have a large number of lifetime sex partners and 2 or more new sex partners in past 6 months. Similarly, significant differences were observed in age at enrollment, country of residence, and the number of lifetime sex partners (either sex) and lifetime female sex partners between seronegative and seropositive MSW. Among MSM, seropositive men were significantly older than seronegative men.

Studies have shown that men who practice same-sex intercourse have higher seroprevalence of vaccine HPV types (HPV-6, -11, -16, and -18) than do those with heterosexual relationship, suggesting that the difference in the anatomic site infected with HPV may be relevant (Stone et al., 2002; Markowitz et al., 2009; Lu et al., 2011; Lu et al., 2012b). Lu and colleagues showed that serum antibody levels and seroprevalence of HPV-6 and -16 were consistently higher in men with corresponding anal HPV infection, regardless of genital coinfection, compared with men with genital HPV infection alone (Lu et al., 2012b). In addition, seroprevalence was higher in MSM than in heterosexual men who had same HPV DNA detected at the same anatomic site (Lu et al., 2012b). Furthermore, HPV6-seropositivity was strongly associated with concurrent detection of anal HPV 6 DNA, but not concurrent detection of genital HPV 6 DNA alone, in both MSW and MSM. Independent associations between HPV 16 seropositivity and anal HPV 16 DNA detection were only observed in MSM. The differential seroprevalence linked to anatomic site-specific HPV DNA detection in men may be explained by the type of epithelium present at each anatomic site. Antigen presentation to the immune system at a mucosal epithelium (*e.g.*, anus, cervix), compared with that at a keratinized epithelium (*e.g.*, shaft, glans in circumcised men), may provide more direct access to the lymphatics and draining lymph nodes where immune responses are initiated, resulting in earlier and stronger antibody responses (Stanley 2010). Furthermore, the histology of the anal canal closely resembles that of the cervix with a transformation zone (Palefsky et al., 1998). The similarity in anatomy of the cervix and the anus suggests that divergent seroprevalence observed in genital and anal HPV positive men likely mirrors gender-related differences in seroprevalence observed in population-based studies. Otherwise, it is likely that the prolonged anal HPV infection harbored by MSM may have contributed to the higher seroprevalence observed in anal HPV-positive MSM compared with anal HPV-positive MSW. It is also likely that repeated anal exposures to HPV among previously infected MSM results in anamnestic responses, leading to the elevated seroprevalence in MSM. In addition, it is possible that direct sexual contact with an infected male partner during receptive anal intercourse allows viral transmission to the squamocolumnar junction of the anal canal where there is little keratinization, resulting in more efficient viral antigen detection by the immune system and stronger antibody responses. In contrast, anal HPV infection

detected in MSW, in the absence of receptive anal sex, is likely acquired via autoinoculation or inoculation through indirect contacts with infected female partners (Hernandez et al., 2008; Goodman et al., 2010; Winer et al., 2010), possibly at the lowest part of the anal canal where the tissue is markedly keratinized, making it less accessible for immune recognition.

Lu and colleagues assessed the risk of incident genital infection and 6-month persistent genital infection with HPV16 in relation to baseline serostatus (HPV-16 VLP-seropositivity) in a cohort of 2,187 men over a 48-month period (Lu et al., 2012a). Genital swabs were collected every 6 months and tested for HPV presence. Incidence proportions by serostatus were calculated at each study visit to examine whether potential immune protection attenuated over time. Overall, incidence proportions did not differ statistically between baseline seropositive and seronegative men at any study visit or over the follow-up period. The risk of incident and 6-month persistent HPV infection was not associated with baseline serostatus or baseline serum antibody levels in the cohort. These findings suggest that baseline HPV seropositivity in men is not associated with reduced risk of subsequent HPV16 acquisition. Thus, prevalent serum antibodies induced by prior infection may not be a suitable marker for subsequent immune protection against genital HPV16 acquisition in men (Lu et al., 2012a).

Recently, Rahman and colleagues have conducted large multinational seroepidemiological surveys on 600 men from Brazil, Mexico and United States of America, using a glutathione S-transferase (GST) L1-based multiplex serologic assay and aiming to assess the seroprevalence of the most detected mucosal HR-HPV (HPV16, 18, 31, 33, 45, 52 and 58), LR-HPV (HPV6 and HPV11), and also the 14 most detected cutaneous HPV types (Rahman et al., 2016a; Rahman et al., 2016b). Overall, seropositivity rates observed in these men were as follows: 14% of them were seropositive for at least one of the seven mucosal high-risk types (HPV-16, -18, -31, -33, -45, -52 and -58), 17.4% for at least one of the low-risk types (HPV-6 and HPV-11) and 65.4% for at least one of the fourteen cutaneous HPV types, respectively. Compared to men aged 18-30 years, men older than 30 years were significantly more likely to be seropositive for any mucosal HR-HPV and any cutaneous HPV type. In addition, current smokers were more likely to be seropositive to mucosal HR-HPV and LR-HPV than never smoker. In contrast, married men were less likely to be seropositive to any mucosal HR-HPV and individual HPV types 18 and 31 than single men. These data indicate that exposure to both the most detected mucosal HR-HPV and LR-HPV types and the cutaneous HPV types is common in men and that seropositivity for these HPV types is associated with older age and the lifetime number of anal sex partners.

The low rates of seroconversion and the low levels of HPV-specific antibodies detected during natural infection likely reflect non-lytic and almost exclusively intraepithelial viral cycle of HPV, even in persistent infection, in which only a very small number of free virus particles are released at the surface of anogenital mucosae (Doorbar et al., 2015), greatly reducing the likelihood of circulating or mucosal APCs encountering HPV antigens.

Serum antibodies to HPV capsids are not thought to clear infections, but they may provide protection against reinfection by the same type, and they can serve as markers for ongoing or previous infections (Carter & Galloway 1997; Gisman et al., 1996; Kirnbauer et al., 1996). In an animal model, immunity to infection was passively transferred by serum (Suzich et al., 1995), suggesting that antibodies protect against reinfection with the same type.

The lack of protection in men observed in the study of Lu and colleagues (Lu 2012a), in contrast to the moderate protection found in a number of studies in women, could be due to several factors including differential gender-related immune response, assay differences, and varying duration of serum antibody presence. Gender differences in seroprevalence have been reported in multiple studies where seroprevalence of oncogenic and nononcogenic HPV was consistently higher among women than in men from the same source population (Svare et al., 1997; Stone et al., 2002; Thompson et al., 2004; Clifford et al., 2007; Markowitz et al., 2009; Lu et al., 2012a). The observed gender-specific immune response may be an indication that HPV infection at keratinized epithelium is less likely to induce immune responses than infection of mucosal epithelium or that heterosexual women may be at higher risk than heterosexual men to have HPV exposures at multiple anatomic sites, mostly mucous membranes such as cervix, oral cavity, and anus, eliciting stronger antibody responses. Furthermore, differences in serologic assays used across studies may have contributed to inconsistent results observed across publications. The VLP-based direct-binding ELISA assay used in the current study measures total type-specific binding IgG antibodies, including neutralizing and nonneutralizing antibodies, whereas the competitive neutralization assay used by Velicer and colleagues measures IgG antibodies that bind to known neutralizing epitopes in HPV viral capsid (Velicer et al., 2009). The inclusion of nonneutralizing antibodies in the present study could have led to overestimation of functional neutralizing antibody levels and biased the association of protection toward the null. Another explanation for the inconsistent results observed could be that prolonged presence of serum antibodies had played a role in conferring protection. The protective effect of serum antibodies reported by Ho and colleagues was only restricted to women who showed high antibody level at 2 or more consecutive visits using a direct ELISA assay (Ho et al., 2002). Conversely, no protection was

detected in other studies of women that determined serostatus only once at the baseline using a similar assay (Viscidi et al., 2004; Trottier et al., 2010).

5. Circulating HPV-specific IgA elicited by natural HPV infection

In women infected by HPV-16 infection, serum HPV-16-specific IgA are induced in a contemporary way to serum HPV-16-specific IgG (Strickler et al., 1997; Saagawa et al., 1998). Bontkes and colleagues showed that there was no correlation between genital and systemic HPV-16 VLP-specific IgA levels measured in samples collected at individual time-points, while there was a good correlation between local and systemic IgG levels (Bontkes et al., 1999). The authors concluded therefore that the VLP-specific IgG antibodies detected locally had likely entered the mucosal surface by transudation from the serum, in contrast to the local IgA antibodies (Bontkes et al., 1999). Furthermore, while HPV-16-specific IgG responses were often found both locally and in the serum at the same time in patients with persistent HPV-16 infection, the HPV-specific IgA responses were never detected simultaneously in paired serum and cervical samples. These findings demonstrate that the systemic IgA immune response to HPV is basically different from the mucosal HPV-specific immune response (Bontkes et al., 1999). Local HPV-16 VLP-specific IgA antibodies appear to be produced locally, while circulating HPV-16 VLP-specific IgG antibodies appear to originate from the systemic compartment (Bontkes et al., 1999). Furthermore, systemic VLP-specific IgA appeared stable, while local VLP-specific IgA declined rapidly after successful treatment and elimination of HPV (Elfren et al., 1996). Furthermore, systemic levels of HPV-specific IgA were correlated with virus clearance and resolution of the lesion (Bontkes et al., 1999). The systemic IgA responses to HPV may be a secondary marker of a successful immune response, mediated by cytokines and induced at the draining lymph nodes rather than by the local mucosae. Thus, the autocrine control of keratinocyte proliferation by the transforming growth factor- β (TGF- β), which inhibits proliferation of both normal and HPV-infected keratinocytes especially at the early stages of HPV infection (Ho et al., 1994), and which is the immunoglobulin class switching factor for IgA (Stavnezer 1995), might contribute to limit the HPV infection. The release of virions will induce an antibody response that will switch to IgA under the influence of TGF- β at the local lymph node (Bontkes et al., 1999). Finally, the systemic IgA responses to HPV were associated with virus clearance and might be an indication of a successful cellular immune response mediated by cytokines (Bontkes et al., 1999).

6. Circulating HPV-specific IgG elicited by natural HPV infection

In the systemic compartment, both HPV-specific monomeric IgA and IgG are commonly detected (Sasagawa et al., 1998), while low levels of HPV-specific IgM have been reported in serum in only one study (Petter et al., 2000). Circulating HPV-specific antibodies responses reflect past-infection with a 50-70% rate of seroconversion in infected women (Dillner et al., 1999; Carter et al., 2000) or persistent viral infection (Sasagawa et al., 1998). Naturally derived HPV-specific IgG antibodies from single-positive sera were highly genotype-specific, while in multi-positive sera, cross-reactive antibodies were observed both within and between $\alpha 7$ (HPV-18 and -45) and $\alpha 9$ (HPV-16, -31, -33, -52 and -58) strains (Scherpernisse et al., 2013). Serum HPV-specific IgA immune responses are much more transient than anti-HPV IgG serum responses (Carter & Galloway 1997; Dillner et al., 1999), and could have a regulatory role to the HPV-specific cellular immune response to clear the virus (Bontkes et al., 1999). The serological IgG immune response to HPV persists over time and is associated with the cumulative number of sex partners over a lifetime and not with the recent number of sex partners (Dillner et al., 1999). Therefore, HPV-specific IgG seropositivity is thought to be a marker of past infection (Dillner et al., 1999). The detection of serum IgG to HPV constitutes the “gold standard” serological test for ongoing or past history of HPV infection or reinfection (Carter & Galloway 1997; Dillner et al., 1999).

6.1. Immunochemical characteristics of circulating natural IgG to L1 capsid protein

In order to evaluate the HPV humoral antibody response, Scherpernisse and colleagues addressed the issue of HPV-specific IgG subclasses and immunochemical characteristics (including avidity, cross-reactivity, neutralizing activity and avidity) induced by natural HPV infection in sera collected from individuals from Netherlands and Rwanda (Scherpernisse et al., 2013b). Serological analyses were carried out *in vitro* using VLP L1-based multiplex immunoassay for HPV -16, -18, -31, -33, -45, -52 and -58, VLP-16 and VLP-18-based inhibition assay and pseudovirion-based neutralization assay.

The most abundant antibody subclass of IgG induced after HPV-16 and HPV-18 infections was IgG1 (78% and 92%, respectively), followed by IgG3 (20% and 6%, respectively). Small amounts of HPV-specific IgG of the IgG2 and IgG4 subclasses were found, 2% and 1% for HPV-16 and 1% and 2% for HPV-18, respectively.

In single type-positive sera for HPV-16 or HPV-18, homologous inhibition of naturally acquired HPV-specific antibodies against a single HPV type reached 94% for HPV-16 and 83% for HPV-18, and 78% to 92% for the other 5 single-seropositive HPV types tested, indicating that high specificity of mono HPV-16 or -18-specific IgG antibodies.

In contrast, sera positive for HPV-16 or HPV-18 and up to 5 other HPV types showed lesser inhibition with VLP-16 or VLP-18 amounting to an average of 75% and 80%, respectively. Furthermore, rates of heterologous inhibition with VLP-16 or VLP-18 of sera multi-positive for 2-6 HPV types were much lower (between 45% to 62%). Finally, naturally acquired HPV-specific IgG antibodies in multi-positive sera appeared less specific than in single type-positive sera.

Single type-positive and multi-positive sera differed also functionally. Indeed, naturally derived HPV-specific antibodies from multi-positive sera showed less neutralizing activity than HPV-specific antibodies of single-positive sera. Thus, 83% and 20% of the single-positive sera showed neutralizing capacity for VLP-16 and VLP-18, respectively, whereas only 16% (VLP-16) and 25% (VLP-18) of the multi-positive sera showed neutralizing capacity. Antibodies that cross-reacted with HPV-31 and HPV-45 showed no neutralizing capacities.

Furthermore, HPV-16 and -18-specific antibodies induced by HPV infections had mainly low-avidity, with an average of 35% and 28% of HPV-16 and -18-specific antibodies of single-positive sera, respectively, remaining detectable after the incubation by chaotropic agent.

Finally, naturally derived antibodies from single-positive sera were highly genotype-specific, while in multi-positive sera, cross-reactive antibodies were observed both within and between $\alpha 7$ (HPV-18 and -45) and $\alpha 9$ (HPV-16, -31, -33, -52 and -58) strains. In addition, HPV-specific IgG1 and IgG3 were found to be the predominant subclasses observed after HPV infection and the number of single-positive sera with neutralizing capacity was higher than the number of neutralizing multi-positive sera. Taken together, Scherpenisse and colleagues concluded that naturally derived HPV-specific antibodies from single-positive samples showed different characteristics in terms of cross-reactivity and neutralizing capacity compared with antibodies from multi-positive sera (Scherpenisse et al., 2013b).

6.2. Circulating natural IgG to early oncogenic proteins

Apart the L1 and L2 capsid proteins synthesized lately and make up the bulk of the virus, early proteins of HPV such as E6 and E7 are also produced in large quantities and

constitute powerful antigens (Kreimer et al., 2017; Lang Kuhs et al., 2017). The early proteins of HPV (particularly E6 and E7) are overexpressed only at some stages of the disease progression, making antibodies specific to these antigen, potential good markers of the progression of HPV-related diseases to cancers (Kreimer et al., 2017; Lang Kuhs et al., 2017).

Serum HPV-16-E6 antibody positivity could be a potential biomarker for oropharyngeal cancer (Kreimer et al., 2013; Kreimer et al., 2017). Up to 80% of patients with HPV-associated oropharyngeal cancer (HPVOPC) have detectable serum HPV-16 antibodies to early HPV antigens (Kreimer et al., 2013; Anderson et al., 2015a; Anderson et al., 2015b; Kreimer et al., 2017).

Thus, in a large cohort of 385,747 participants recruited from 1992 to 2000 from 10 European countries, HPV-16-E6 seropositivity was present in 35% of patients with oropharyngeal cancer and 0.6% of controls (OR, 274; 95% CI: 110–681), but was not associated with other cancer sites (Kreimer et al., 2013). The increased risk of oropharyngeal cancer among HPV-16-E6 seropositive participants was independent of time of diagnosis and was observed more than 10 years before diagnosis. The all-cause mortality ratio among patients with oropharyngeal cancer was 0.30 (95% CI, 0.13–0.67), for patients who were HPV-16-E6 seropositive as compared with those being seronegative (Kreimer et al., 2013).

In another large Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) conducted in United States and including 155,000 participants recruited between 1993 and 2001, the seroprevalence of HPV-16-E6 antibodies was 42.3% in patients with oropharyngeal cancer and 0.5% of control subjects (Kreimer et al., 2017). The estimated 10-year cumulative risk of oropharyngeal cancer in HPV-16-E6 antibodies seropositive men was at least 4 times higher than in HPV-16-E6 seropositive women (6.2%; 95% CI: 1.8–21.5% versus 1.3%; 95% CI: 0.1–15.3%; respectively). Furthermore, this 10-year cumulative risk was at least 155-fold higher than in HPV-16-E6 seronegative individuals (6.2%; 95% CI: 1.8–21.5% versus 0.04%; 95% CI: 0.03%–0.06%; respectively) (Kreimer et al., 2017). Taken together, 35% of European (Kreimer et al., 2013) and 42% of American (Kreimer et al., 2017) patients diagnosed with oropharyngeal cancer between 1990 and 2010 were HPV-16-E6 seropositive, with stable antibody levels during annual follow-up for up to 13 years prior to diagnosis. Tumor analysis indicated that the sensitivity and specificity of HPV-16-E6 antibodies were exceptionally high in predicting HPV-driven oropharyngeal cancer (Kreimer et al., 2013; Kreimer et al., 2017).

The majority (64%) of patients with HPVOPC have antibodies against HPV-16 E2 (Anderson et al., 2015a; Anderson et al., 2015b).

In cervical disease, anti-E1 antibodies have been reported to have a low prevalence (10% and 0.3% in invasive cervical cancer and healthy controls, respectively) (Combes et al., 2014). In contrast, similarly to oropharyngeal cancer, early HPV antigens (E4, E6 and E7)-derived antibodies could constitute biological markers with high sensitivity and specificity for high-grade cervical intraepithelial neoplasia (CIN 1-3) and cervical cancer in women (Salazar-Piña et al., 2016; Jin et al., 2018).

Salazar-Piña and colleagues evaluated the clinical performance of a slot blot assay detecting serum HPV-16 E4, E7 and VLPs-L1 antigens derived antibodies as serological markers for high-grade precancerous lesions progression and cervical cancer (Salazar-Piña et al., 2016). The system was validated with sera from a female population (n=485) aged 18 to 64 years referred to the dysplasia clinic at the General Hospital in Cuautla, Morelos, Mexico. High prevalence of anti-E4 (73%) and anti-E7 (80%) antibodies was observed in women diagnosed with cervical cancer (Salazar-Piña et al., 2016). Furthermore, seropositivities to 1, 2, or 3 antigens derived antibodies showed significant associations of increasing magnitude with cervical cancer (ORs: 12.6, 19.9, and 58.5, respectively). The highest association with cervical cancer was observed when the analysis was restricted to only anti-E4+E7 antibodies (OR=187.7). The best clinical performance to discriminate cervical cancer from CIN 1 to 3 was the one for the combination of anti-E4 and/or anti-E7 antibodies, which displayed high sensitivity (93.3%) and moderate specificity (64.1%), followed by anti-E4 and anti-E7 antibodies (73.3% and 80%; 89.6% and 66%, respectively). In addition, the sensitivity of anti-E4 and/or anti-E7 antibodies was high at any time of sexual activity, suggesting that these antibodies can be biomarkers for the early detection of cervical cancer. The sensitivity of anti-E4 antibodies was low (<10%) when the time of sexual activity was <10 years, and it increased up to 100% in relation to the time of sexual activity, suggesting that anti-E4 antibodies can be useful as HPV exposure markers at early stages of the disease (Salazar-Piña et al., 2016).

Similar observations have been reported in Korean women in whom the prevalences of nine serum antibodies (anti-E6, anti-E7 and anti-L1 antibodies of HPV types 16, 18, and 58) were evaluated (Jin et al., 2018). The frequencies of all types of anti-HPV antibodies were higher in the CIN stages and cervical cancer than in normal women, and those of anti-HPV-16 E6 and E7, anti-HPV-18 E6 and E7, and anti-HPV-58 E7 antibodies were higher in the cervical cancer group than in the CIN stages. The frequencies of antibodies against HPV-16, -18, and -58 E7 tended to increase with increasing severity of cervical lesions. However, there were few differences in the frequencies of antibodies against the L1 antigens of HPV-16, -18 and -58 in cervical cancer versus CIN stages.

Recently, Ewaisha and colleagues developed programmable protein arrays (NAPPA) that display the proteomes of two low-risk HPV types (HPV6 and 11) and ten oncogenic HR-HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52 and 58), in order to detect circulating HPV-specific IgG antibodies in sera from women with CIN 0/I (n=78), CIN II/III (n=84), or invasive cervical cancer (n=83) (Ewaisha et al., 2017). While L-specific antibodies were detected in serum from women in all three groups of cervical disease, antibodies against any early HPV proteins were, as expected, more prevalent in invasive cervical cancer and CIN II/III than women with CIN 0/I. Antibodies to any early HPV proteins were detected less frequently in women with CIN 0/I (23.7%) than women with CIN II/III (39.0%) and invasive cervical cancer (46.1%, $p < 0.04$). Of the antibodies to early HPV proteins, anti-E7 antibodies were the most frequently detected (6.6%, 19.5%, and 30.3%, respectively), while anti-E6 antibodies were less frequently detected (3.9%, 9.8%, and 15.8%, respectively). The least frequently detected antibodies were directed to E1 and E2 in CIN 0/I (1.3%) and to E1 in CIN II/III (1.2%) and invasive cervical cancer (7.9%). HPV-16-specific antibodies correlated with HPV-16 DNA detected in the cervix in 0% of CIN 0/I, 21.2% of CIN II/III, and 45.5% of invasive cervical cancer. A significant number (29 - 73%) of E4, E7, L1, and L2 antibodies showed cross-reactivity between HPV types (Ewaisha et al., 2017). Finally, 20 - 46% of patients with CIN and invasive cervical cancer had a broad range of antibodies to HPV early proteins in their sera and these biomarkers correlated with disease severity. HPV NAPPA provided a valuable high-throughput tool for measuring the breadth, specificity, and heterogeneity of the serologic response to HPV in cervical disease.

As cervical disease progresses towards malignancy, infectious viral particle production becomes limited to a small area near the surface of the cervical epithelium (Griffin et al., 2012). E4 plays a role in viral synthesis and possibly viral release (Doorbar et al., 2013). The expression of E4 in CIN II and III is restricted to this subset of cells and is generally lost in invasive cervical cancer (Doorbar et al., 2013). Expression of E4 protein in tissue has been proposed as an early detection marker (Griffin 2012), but specific serum antibodies may provide a more convenient detection method. Indeed, E4-specific antibodies develop early in disease progression, with 16%-17% (Ewaisha et al., 2017) to 29%-34% (Pedroza-Saavedra et al., 2000), prevalence in both CIN II/III and invasive cervical cancer, which is consistent with the predicted level of E4 expression especially in CIN II/III.

Viral integration into the host genome, with loss of E2 expression, is a frequent hallmark of HPV-associated cancers, leading to derepression of E6 and E7 expression. Since E2 antigen is expressed in CIN II/III (Xue et al., 2010), E2-specific antibodies were detected in both CIN II/III (4.9%) and at significantly higher frequency in invasive cervical cancer

(14.5%) but were at low prevalence (Ewaisha et al., 2017). In other series, E2-specific IgG antibodies have been reported in 24% of women with CIN I-III (compared to 13% of healthy controls) (Marais et al., 1997) and in 12% of women with invasive cervical cancer (compared to 2% of healthy controls) (Combes et al., 2014). These features contrast with the high prevalence of anti-HPV-16 E2 antibodies in HPVOPC (Anderson et al., 2015a; Anderson et al., 2015b), suggesting that the oropharyngeal and cervical tumor sites may have significant differences in viral integration and expression of E2 antigen, or exposure of E2 antigen to B cells.

In conclusion, antibodies to both HPV-16 E6 and E7 proteins have been detected at low levels in both serum and cervical-vaginal secretions of invasive cervical cancer patients (Meschede et al., 1998; Bierl et al., 2005). Their levels increase with cervical disease progression but they are not detectable in a subset of patients with cervical cancer (Stanley 2003; Reuschenbach et al., 2008; Luevano et al., 2010; Gutierrez-Xicotencatl et al., 2016). They develop later in the course of invasive cervical cancer, and are correlated with disease outcome (Ravaggi et al., 2006; Silins et al., 2002; Gutierrez-Xicotencatl et al., 2016). Studies of sera collected prior to the diagnosis of cervical cancer have shown that the presence of E6 and E7-specific antibodies is associated with an increased relative risk (RR=2.7) for cervical cancer, and can be detected, albeit infrequently, up to 5 years prior to diagnosis (Lehtinen et al., 2003). The percentage of women with false negative serology is dependent on the method of antibody detection (Achour et al., 2009; Zumbach et al., 2000; Waterboer et al., 2005; Luevano et al., 2010; Combes et al., 2014). All in all, the antibodies against E7 proteins appeared to have the highest potential of the anti-HPV antibodies tested as markers for detecting cervical lesions (Ewaisha et al., 2017; Jin et al., 2018).

Antibodies elicited by early HPV antigens, mainly E7, could represent interesting reliable specific and sensitive markers for the HPV diseases profiling and for monitoring the disease progression to HPVOPC (Kreimer et al., 2013; Kreimer et al., 2017) as well cervical cancer (Ewaisha et al., 2017). However, the signal intensity of antibody binding on the arrays (Ewaisha et al., 2016) and on RAPID ELISA (Anderson et al., 2015a; Anderson et al., 2015b) are consistently weaker in cervical disease than in HPVOPC. Additionally, despite tissue expression of the oncoproteins E6 and E7 in invasive cervical cancer, E6/E7 specific serum antibodies may be detected in less than half of the patients (Stanley 2003; Reuschenbach et al., 2008; Luevano et al., 2010), while they are detected in up to 75% of HPVOPC cases (Anderson et al., 2015a). Anti-E6 antibodies have been detected in HPVOPC and cervical cancer cases years prior to the establishment of a clinical diagnosis (Lehtinen et al., 2003; Kreimer et al., 2013), suggesting these may be useful for early detection. The antibodies

response to the early HPV proteins in high grade pre-invasive cervical lesions, however, has been difficult to detect in previous studies (Lehtinen et al., 2003; Stanley 2003; Luevano et al., 2010) or at low frequencies (Ewaisha et al., 2017). The presence of both viral DNA and viral oncoproteins in HPVOPC tumors suggest that both cancer sites have similar pathogenetic mechanisms (Gillison et al., 2008). Therefore, it is likely that the close proximity of lymphoid tissue in the tonsils results in a more potent immune induction in HPVOPC compared with cervical disease.

7. Circulating HPV-specific IgM elicited by natural HPV infection

HPV-specific IgM have been poorly studied. Serum HPV-specific IgM were detected by indirect VLP-based ELISA in one report (Petter et al., 2000). While HPV-specific IgA responses were comparable to HPV-specific IgG, IgM responses to HPV were low. Specific IgM to high risk HPV (-16, -18 and -31) were detected in 12% of HIV-infected women, in 4% HIV-negative CIN/cancer women and in 1% of HIV-negative controls; IgM to low risk HPV (-6 and -11) were detected in 15% of HIV-infected women, in 0% HIV-negative CIN/cancer women and in 3% of HIV-negative controls. HPV-specific IgM reactivity was found to be far less type-specific than HPV-specific IgA or IgG, e.g. HPV-specific IgM positive sera reacted in 44% with both HPV-6 and -16; in comparison, this percentage is much higher than observed and mentioned above with IgG and identical HPV-6 and -16 VLPs (12%). The frequency and type specificity of HPV-specific IgM isotype was generally low, and therefore there was no evidence of a time correlation between HPV IgM seroconversion and recent HPV infection, as it is generally known from other infections. The role of HPV IgM isotype in clinical practice remains to be determined in future studies.

8. Mucosal HPV-specific antibodies elicited by natural HPV infection

To our knowledge, studies on HPV-specific mucosal immune response remain scarce, that appears surprising for a strictly mucosal infection. Only few studies have been reported on the mucosal humoral immune response to HPV, summarized in the Table 2 (*Annexe 1*).

Natural B cells-derived humoral responses to HPV has been described in two mucosal compartments, including the cervicovaginal secretions (Wang et al., 1996) and the saliva (Marais et al., 2001). It has been demonstrated that local antibodies directed against HPV are predominantly of the IgA and IgG classes, while mucosal HPV-specific IgM could never be evidenced (Wang et al., 1996).

In the cervicovaginal compartment, HPV-specific IgA, secretory-IgA and IgG, but not IgM, have been evidenced at low levels (Wang et al., 1996; Sasagawa et al., 2003; Mbulawa et al., 2008). Cervicovaginal HPV-specific S-IgA are locally produced (Wang et al., 1996) and are thought to reflect current genital HPV infection (Sasagawa et al., 2003) or HPV-related cervical lesions (Hagensee et al., 2000). Cervicovaginal HPV-specific monomeric IgA may be debris of genitally produced S-IgA or serum-derived transudative IgA (Hocini et al., 1995). In healthy women without cervical pathology IgA against E2, E7, L1, and L2 from HPV-16 was observed in 15–32% of the secretions (Dillner et al., 1989; Dillner et al., 1993; Dreyfus et al., 1995). IgA antibodies against HPV capsids from HPV-6, -11, -16, -18, -31, -33 and -35 were detected in cervical mucus from patients with cervical neoplasia and controls (Wang et al., 1996). In other studies, local antibodies have been detected against E2, E7, L1, and L2 of HPV 16 in cervical secretions and in cervicovaginal washings of women with cervical neoplasia, patients with condylomata and women with normal cervical cytology (Dillner et al., 1989; Dillner et al., 1993; Dreyfus et al., 1995; Veress et al., 1994). IgA against HPV-16 E2 was found in 49% of the cervical secretions from patients with CIN (Dreyfus et al., 1995). In another study in patients with condylomata, IgA against HPV-16 E2, E7, L1, and L2, were found in 10–62% of the cervical secretions (Dillner et al., 1993), which is at least remarkable since condylomata are generally not associated with HPV16.

Cervicovaginal HPV-specific monomeric IgG are mainly transudative from circulating IgG, while the possibility of an active mucosal cervicovaginal secretion of HPV-specific IgG may be induced by HPV particles uptake from the genital mucosal and in fine by the development of cervical lesions (Tjiong et al., 2000; Tjiong et al., 2001; Sasagawa et al., 2003). Local IgG against HPV-16 E2 were reported in 46% of patients with CIN and in 15% of healthy controls (Dreyfus et al., 1995). Whatever and interestingly, cervicovaginal HPV-specific IgG are 2.5 more abundant than cervicovaginal IgA and S-IgA to HPV (Hagensee et al., 2000), as previously demonstrated for HIV infection (Bélec et al., 1995). Indeed, a general predominance of IgG over IgA exists in cervicovaginal secretions (Hocini et al., 1995; Quesnel et al., 1997; Mestecky & Fultz 1999). These findings point the critical interest to evaluate the HPV-specific immune IgG response in cervicovaginal secretions as marker of ongoing or previous HPV infections.

By luminescence immunoassay, Hagensee and colleagues reported IgA, IgG, and S-IgA antibodies to HPV-16 capsids in cervical samples in 11%, 24%, and 9%, respectively, in college-aged women who were examined at 4-month intervals (Hagensee et al., 2000). Cervical IgG antibodies were most strongly associated with HPV-16 DNA detected within the previous 12 months (odds ratio, 3.3). Secretory-IgA (i.e. cervical IgA with positive secretory

piece) was most strongly associated with detection of a squamous intraepithelial lesions 4–8 months earlier (odds ratio, 6.4). As with serum HPV-16 antibodies, there appears to be a several-month delay between cervical HPV infection and detection of cervical antibodies.

HPV-specific antibodies can be detected in the unstimulated saliva from nonvaccinated individuals (Marais et al., 2000; Marais et al., 2001; Passmore et al., 2007). In South African women with cervical neoplasia, Marais and colleagues detected anti-VLP16 IgG and IgA antibodies by ELISA in oral fluid (Marais et al., 2001). Anti-VLP-16 IgA and IgG antibodies were detected in saliva from 54.3% and 34.7%, respectively, in women with cervical neoplasia, compared with 8% and 13.3%, respectively, in controls. Only a small percentage of oral IgA antiVLP-16 responses were found to correlate with the serum IgA responses. This supports the finding that only small amounts of salivary IgA are derived from the circulation and the remainder is synthesized locally in salivary glands (Mestecky et al., 1987). Oral IgA antibody testing could be useful as a non-invasive screening method for women with cervical neoplasia and for estimating the mucosal antibody response to HPV vaccines.

The importance of local immunity is even more stressed since many studies currently focus on the development of HPV vaccines both prophylactic and therapeutic. It is reasonable to assume that the efficacy of vaccines against this locally transmitted infection can be best assessed by parameters of local immunity. It will be tempting to find out whether local HPV-specific IgG and/or S-IgA are the main neutralizing and protective antibodies against HPV infections in the female and male genital tract, in the oral cavity and surely at the anal mucosa level. Therefore, it will be important to continue the study of the mucosal cellular and humoral immune responses against HPV.

9. Naturally acquired humoral immunity to HPV: Correlates of protection

9.1. Adult female population

Several studies have tried to provide relevant answers to the interrogations surrounding the naturally acquired humoral immunity as a protective factor against subsequent HPV infections or as the potential marker for the disease progression. So far, several points are still debated (Carter et al., 2000; Dillner et al., 2007; Malik et al., 2009; Safaeian et al., 2010; Wentzensen et al., 2011; Moller et al., 2013; Castellsagué et al., 2014; Beachler et al., 2016; Safaeian et al., 2018).

Considering the facts that the development of HPV-induced seroconversion is not the rule after natural infection (Carter et al., 2000), that it often takes several months before

seroconversion can be detected (Carter et al., 2000), and that most transient HPV infection do not elicit a detectable antibody response (Carter et al., 2000), Dillner and colleagues suggested that HPV antibodies do not play an important role in the protection or clearance of HPV infections but only represent a serological scar or at most a marker of exposure (Dillner et al., 2007). However, more recent studies have highlighted some evidences about the protecting role played by the naturally acquired HPV-type specific antibodies.

In a large community-based, randomized HPV-16/-18 vaccine trial conducted in Costa Rica and including more than 3,000 healthy women (18–25 years) in the control arm, Safaeian and colleagues reported reduction of new incident HPV-16 infections in women naturally seropositive for HPV-16 or HPV-18 antibodies (Safaeian et al., 2010). The seroprevalence of HPV-16 and HPV-18 was 25% at enrollment in the unvaccinated women. After the follow-up period, a significant reduction of new homologous HPV type infections was observed in women who exhibited the highest HPV-16 and HPV-18 antibody titers. The naturally acquired protection conferred by HPV-16 and HPV-18 type-specific serum antibodies was 50% and 64%, respectively.

Another major study was conducted by Castellsagué and colleagues on the large multinational randomized so-called PATRICIA (Papilloma Trial against Cancer In Young Adults) trial evaluating the HPV-16/-18 vaccine in which more than 8,000 women (15–25 years of age) constituted the control arm (Castellsagué et al., 2014). Based on VLP-based ELISA that measures a broad spectrum of neutralizing and non-neutralizing HPV-16/-18 L1-epitopes specific antibodies, the association of HPV-16 and HPV-18 serum antibody levels and the risk to develop new homologous (same type) HPV infections and cervical lesions was evaluated. In this large population-based study, Castellsagué and colleagues highlighted that high titers of HPV-16 serum antibodies, but not HPV-18 serum antibodies, were significantly associated with a lower risk of incident and persistent homologous type infection, and also with a lower risk of atypical squamous cells of undetermined significance (ASCUS) and also low- and high- grade cervical intraepithelial neoplasia (CIN 1–3). When comparing with HPV-16-seronegative women, a 36% (95% CI: 22–47%) reduction of new incident HPV-16 infections was observed in women naturally seropositive for HPV-16 antibodies. Moreover, the natural protection elicited by HPV-16 serum antibodies significantly increased proportionally to the increase of the antibody titer, reaching 66% (95% CI: 46–79%) for women with the highest HPV-16 antibody titers (Castellsagué et al., 2014).

According to these data, a recent pooled analysis based on serological results from the control arms of the two above mentioned large vaccine trials concluded for an association with partial protection from future HPV-16 and HPV-18 infections and high HPV-16 and

HPV-18 serum antibody titers (Safaeian et al., 2018). Quite similarly to that reported by Castellsagué et al. (Castellsagué et al., 2014) and Safaeian et al. (Safaeian et al., 2010), Wentzensen and colleagues also reported a significant naturally acquired protection (46%) elicited by HPV-16, but not by HPV-18 type-specific serum antibodies in adult women (mean age, 37 years) also living in Costa Rica (Wentzensen et al., 2011). The slight difference observed, particularly for HPV-18 acquired immune protection, could be explained by the different serological assays used in these studies. Indeed, Castellsagué et al. and also Safaeian et al. used a VLP-based ELISA assay measuring neutralizing and non-neutralizing antibody titers while Wentzensen et al. used a competitive luminex immunoassay (cLIA) measuring only neutralizing antibody levels (Safaeian et al., 2010; Wentzensen et al., 2011; Castellsagué et al., 2014).

Along with the protective effect afforded by the high titers of HPV antibodies in certain women, Malik and colleagues showed that persistent antibody response following natural infection in women constituted a strong barrier against subsequent infection caused by related and also unrelated HPV types (Malik et al., 2009).

More recently, a meta-analysis conducted by Beachler and colleagues (Beachler et al., 2016), including more than 24,000 individuals from 18 countries (including the two above mentioned vaccine trials) that examined HPV natural immunity confirmed evidences of type-specific protection against subsequent infection for a combined measure of HPV-6/-11/-31/-33/-35/-45/-52/-58 in female subjects. A significant protection could be observed in female subjects for subsequent infection with HPV-16 (pooled RR: 0.65; 95% CI/ 0.50–0.80) and HPV-18 (0.70; 95% CI: 0.43–0.98). According to these observations, Beachler and colleagues suggested that naturally acquired HPV-type specific antibodies likely provide modest protection against subsequent infections in women (Beachler et al., 2016).

Taken together, naturally induced anti-HPV serum antibodies in women is associated with host immune protection against subsequent HPV acquisition and progression to precancerous lesions and cancers.

9.2. Adult male population

Concerning the ability of the naturally, HPV induced humoral immunity to protect against subsequent infection; several serological population-based studies conducted in men don't reached similar conclusions than that reported for women. Indeed, large serosurveys aiming to assess the potential protection of naturally acquired HPV-type specific antibodies in heterosexual men and both HIV-negative and HIV-positive MSM reported high titers and

long persistency of serum HPV-specific antibodies (particularly HPV-16 antibodies) similar to that found in female populations, but no evidence for any protective effect played by these naturally induced HPV antibodies on subsequent anogenital HPV infection could be observed (Lu et al., 2012; Mooij et al., 2014a; Beachler et al., 2016; Pamnani et al., 2016; Beachler et al., 2018). Another relevant characteristic of natural HPV-antibody response in men pointed out by these studies was the different seroprevalence rates elicited by genital and anal HPV infection. Indeed, the higher rates of serum HPV-antibodies were detected in MSM, especially those positive for HPV DNA at the anal site, than in heterosexual men who were positive for HPV DNA only at the genital site (Edelstein et al., 2011; Lu et al., 2012; Mooij et al., 2014b; Van Rijn et al., 2014; Zou et al., 2016; Poynten et al., 2018). These data are in accordance with a possible influence played by the anatomic site where the infection occurred on the associated immune response in men, as infection at mucosal epithelia such as the anal canal may induce stronger immune response compared to infection at keratinized epithelia such as the genital skin (Lu et al., 2012; Heiligenberg et al., 2013; Vriend et al., 2013). However, since the naturally acquired serum antibodies elicited by HPV infection do not confer protection in male subjects, men and especially MSM are therefore highly susceptible to recurrent HPV infections compared to women.

10. Naturally acquired humoral immunity to HPV in HIV-infected individuals

Human immunodeficiency virus (HIV) infection is an independent risk factor strongly associated with the occurrence of HPV new infection, recurrence, persistence and also the progression to anogenital cancers in both men and women (Mboumba Bouassa et al., 2017; Mboumba Bouassa et al., 2018). So far, it is not clear whether HIV influences the pathogenesis of HPV-related diseases directly, through molecular interaction with HPV genes, or through its effects on the host immune system. Nevertheless, as HIV hampers the entire host immune system by inducing the depletion of T helper CD4⁺ cells, HIV infection may have a relevant impact on the immune response against HPV (Marais et al., 2000; Dreyer et al., 2018).

Studies evaluating the impact of HIV infection on the B-cell derived HPV immune response are scarce. To our knowledge, three main serological surveys have evaluated the impact of HIV infection on circulating humoral immune responses to HPV and therefore on the potential naturally acquired HPV protection, by using HPV-16 and HPV-6/11/16/18 and 31 VLP-based ELISA assays (Petter et al., 2000; Viscidi et al., 2003; Viscidi et al., 2005).

Petter and colleagues observed extraordinarily significant high titers of HR-HPV L1-specific antibodies (HPV-16, -18 and/or -31) in most (58%) of HIV-positive women compared to HIV-negative control women (19%), suggesting a high lifetime cumulative exposure to HPV in HIV-infected women and increased expression of capsid proteins due to cellular immunodeficiency (Petter et al., 2000).

Viscidi and colleagues found a globally low rate of serum HPV-specific IgA prevalence in study women, including 2,008 HIV-infected and 551 HIV-negative women, compared to that commonly found for serum IgG (7% versus 51%) (Viscidi et al., 2003). However, these low rates of IgA seropositivity were found to be strongly associated with cervicovaginal HPV-16 DNA shedding and cytological abnormalities, mostly in HIV-infected women who were more likely to progress to high-grade cervical abnormalities than HIV-negative women (relative hazard, 2.2; 95% CI: 1.2–4.2) (Viscidi et al., 2003). As IgA seropositivity was significantly higher in HIV-infected women than in HIV-negative women (7.7% versus 4.9%, respectively), these findings raise the possibility that serum IgA response to HPV-16 may be a marker of persistent viral replication in HIV-infected women, but not in HIV-negative women. In addition, the risk of incident infection with non-16-related HPV types was increased in HPV-specific IgA-seropositive women (relative hazard, 1.8; 95% CI: 1.3–2.6), compared to seronegative women (relative hazard, 2.2; 95% CI: 0.9–5.4), but there was no difference in the risk of incident HPV-16 infection or for infection with genetically related HPV types. These observations may reflect the possible partial type-specific or clade-specific immunity conferred by the serum IgA response against HPV-16 capsid proteins (Viscidi et al., 2003).

In another study from Viscidi and colleagues, the association between seropositivity to VLP of HPV types 16, 18, 31, 35, or 45 and subsequent cervical HPV infection was examined in 829 women with HIV and 413 risk-matched HIV-negative women (Viscidi et al., 2005). No statistically significant differences between HPV-seropositive and HPV-seronegative women could be evidenced in the risk of a new infection with the homologous HPV type, with the exception of a reduced risk of HPV-45 infections, 4.5 years beyond the baseline serology measurement in HIV-positive women (hazard ratio, 0.21; 95% CI, 0.05-0.89). Among HIV-negative women, HPV seropositivity was not associated with a statistically significant reduced risk of infections with related viruses in the HPV-16, HPV-18, or "other" HPV groups. Among HIV-positive women, HPV seropositivity was associated with a slightly increased risk of infection with group-related viruses, but the differences were only statistically significant for infection with HPV-16 group viruses (hazard ratio, 1.6; 95% CI, 1.1-2.3) in HPV-18-seropositive women and for infections with "other" HPV group viruses in

HPV-31-seropositive women (hazard ratio, 1.45; 95% CI, 1.0-2.0). The lack of a protective immune effect from natural infection was most likely due to the low level of antibody elicited by natural HPV infection and/or the potential for reactivation of HPV, especially in HIV-positive women (Viscidi et al., 2005).

Additional and more detailed features of HIV infection on HPV induced humoral response have been highlighted by Marais and colleagues in a cohort of high-risk female sex workers in South Africa (Marais 2009). Shortly after HIV-1 seroconversion a significant increase in multiple (>1) HPV infection (OR 4.0, 95% CI 1.3-11.9) was observed compared with HIV-1 seronegative (HIV-negative) women and certain changes in HPV type infection. HIV-1 seroconversion resulted in a reduced prevalence of serum HPV-16 IgA and cervicovaginal IgA and IgG but an increased prevalence of serum HPV-16 IgG. According to these findings, Marais and colleagues suggested that HIV infection caused a reduced ability to produce cervical HPV-16 antibodies but a continued ability to generate serum HPV-16 IgG antibodies (Marais et al., 2009).

Finally, Namujju and colleagues in Ugandan women infected with HIV reported an increasing risk for multiple HR-HPV seropositivity in case of HIV infection (Namujju et al., 2011). Thus, sera were analyzed for antibodies to eight HR-HPV types of the α -7 (18/45) and α -9 (16/31/33/35/52/58) species of HPV by using a multiplex serology assay. In these women, Namujju and colleagues show that seropositivity for greater than one HR-HPV type was as common (18%) as for a single type (18%). HIV-positive women had higher HPV-16, HPV-18 and HPV-45 seroprevalences than HIV-negative women. In multivariate logistic regression analysis, age (>30 years) and level of education (secondary school and above) reduced the risk, whereas parity (>5) and HIV-positivity increased the risk for multiple HR-HPV seropositivity. However, in stepwise logistic regression analyses, HIV-status remained the only independent, stand-alone risk factor (OR, 1.7, 95%; CI 1.0-2.8). On the other hand, the risk of HPV-16 or HPV-18 seropositive women, as compared to HPV-16 or HPV-18 seronegative women, for being seropositive to other HR-HPV types was not significantly different when they were grouped by HIV-status. In conclusion, seropositivity to HPV-16, HPV-18 and to non-vaccine HR-HPV types was common in Ugandan women, suggesting that there is little natural cross-protective immunity between these HPV types (Namujju et al., 2011). HIV-positivity was an independent, stand-alone, albeit moderate risk factor for multiple HR-HPV seropositivity (Namujju et al., 2011).

Taken together, these results support the maintenance of HPV-specific seroreactivity in HIV-infected women, suggesting thus that although HIV infection does not hamper

significantly the naturally acquired humoral response against HPV infection in women and its possible protective effect.

As for women, HIV-infected men and particularly HIV-infected MSM are at very high risk for HR-HPV infection (Mboumba Bouassa et al., 2018). In MSM, HIV infection constitutes an independent risk factor strongly associated with the increased incidence of anal infection with multiple HR-HPV types, HPV DNA persistence and reactivation (Mboumba Bouassa et al., 2018). However, it still unclear whether HIV-infection hamper the naturally acquired humoral immunity to HPV, that could participate to the high susceptibility of HIV-infected MSM to anal HPV infection with multiple types. In order to address this issue, Alberts and colleagues conducted a seroepidemiological study using an HPV-16/-18 L1 Luminex®-based multiplex serology assay in 415 HIV-negative and 205 HIV-positive MSM reporting recent receptive anal intercourse (Alberts 2015). Both HPV-16 and HPV-18 seroprevalences were significantly higher in HIV-positive than in HIV-negative MSM (65% versus 31% for HPV-16 L1 seropositivity; and 51% versus 28% for HPV-18 seropositivity). After adjustment for important risk factors, HIV appeared to be a strong predictor of both HPV-16 (adjusted odds ratio [aOR], 2.80; 95% CI: 1.75–4.49%) and HPV-18 (aOR, 1.78; 95% CI: 1.11–2.85) seropositivity. These observations indicate that HIV infection constitute an important risk factor for both HPV-16 and HPV-18 seropositivity among MSM reporting recent high-risk sexual behaviors (Alberts et al., 2015).

Mooij and colleagues reported similar findings, with both HPV-16 and HPV-18 seropositivity being higher in HIV-infected than HIV-negative MSM (62.7% versus 37.1% for HPV-16 L1 seropositivity; and 42.5% versus 29.1% for HPV-18 seropositivity) (Mooij et al., 2013). Similar features were also observed for HPV types 31, 33, 45, 52 and 58 (Mooij et al., 2013). In multivariate analyses, HPV seropositivity was associated with HIV infection [aOR = 2.1; 95% confidence interval, 1.6-2.6]. In multivariate analyses stratified by HIV status, increasing age and number of lifetime male sex partners were significantly associated with HPV seropositivity in HIV-negative, but not HIV-infected MSM. Finally, seroprevalence of HR-HPV types was high among unvaccinated MSM (Mooij et al., 2013). Furthermore, HIV infection appeared as a strong and independent determinant for HPV seropositivity, likely because of increased persistence of HPV infection in HIV-infected MSM (Mooij et al., 2013).

Mooij and colleagues also assessed HPV seroconversion following anal and penile HPV infection in HIV-negative and HIV-infected MSM (Mooij et al., 2014b). Incident or persistent anal HPV infection, but not penile infection, was an independent determinant of HPV seroconversion in HIV-negative MSM, not in HIV-positive MSM, supporting that HPV

seroresponse may vary per anatomic site (different responses between anal and penile infections) and that persistent HPV infections are more likely to elicit a detectable humoral immune response (Mooij et al., 2014b). Finally, HPV seropositivity among MSM appears to be largely associated with anal HPV infection, irrespective of additionally infected anatomical sites such as penile shaft, and oral cavity (Mooij et al., 2014b; van Rijn et al., 2014).

As for HIV-negative men, no evidence of any protective effect played by naturally induced HPV-specific antibodies on subsequent anal or penile HPV infection could be evidenced, although high rates of HPV-specific antibodies seroprevalence is the rule in HIV-infected MSM (Mooij et al., 2013; Mooij et al., 2014a).

11. From naturally acquired humoral immunity to HPV prophylactic vaccine

Natural infections in animals show that neutralizing antibody to the virus coat protein L1 is protective suggesting that this would be an effective prophylactic vaccine strategy (Shope et al., 1937; Stanley 2008). The current prophylactic HPV VLP vaccines are delivered via the intramuscular routes circumventing the intra-epithelial immune evasion strategies. These vaccines generate high levels of antibody and both serological and B cell memory as evidenced by persistence of antibody and robust and rapid recall responses. However, there is no immune correlate, i.e. there is no defined threshold for the humoral anti-HPV response (amount of secreted antibodies) beyond which vaccinated individuals are systematically protected against HPV infection.

11.1. The challenging issues of HPV prophylactic vaccine design

The first challenge for an HPV vaccine is to demonstrate protective efficacy through a systemic immune response for a virus that enters only via the mucosal route and remains localized there. Furthermore, an ideal prophylactic vaccine should provide a broader protection against all the HR-HPV types and also those classified as possible carcinogenic to eliminate the need for secondary prevention and HPV-related cancers. Thus, the HPV prophylactic vaccine should theoretically induce strong, sterilizing and generalized immunity both in the systemic and the mucosal compartments, as the mucosal location of the infection lack powerful lymphoid inductor site (particularly for female and male genital tracts). To ensure protective immune responses throughout sexually active life, the HPV prophylactic vaccine should also induce high and sustained level of neutralizing antibodies both in mucosal and serum compartments as long as the vaccinated individual remains sexually active.

Furthermore, the ideal HPV vaccine should have both a prophylactic and a curative effect. Indeed, the HPV vaccine should be effective in protecting uninfected persons from possible primary infection, but it should also be able to eliminate a pre-existing HPV infection. As the already HPV-infected cells and also precancerous lesions have been found to be mostly cleared by the T-cells mediated cellular response rather than the B-cells derived antibody response, the HPV vaccine should be able to stimulate both the B-cells derived antibody and the T-cells mediated cellular responses. In addition, HPV vaccine should be able to strengthen a naturally acquired anti-HPV immune response pre-existing in individuals previously exposed to HPV. Finally, the ideal HPV vaccine should be use without age restriction (Roden & Stern, 2018).

11.2. Current HPV prophylactic vaccines

Although the elaboration of the ideal, totipotent HPV vaccine is uncertain, the sustained advances in biotechnology in these last three decades have allowed to build three prophylactic HPV vaccines currently used as primary prevention against HPV infection and related diseases, and several other broad spectrum and therapeutic vaccine candidates are also well advanced in their pre-clinical and clinical evaluation process (Signorelli et al., 2017; Cheng et al., 2018; Dadar et al., 2018; Roden & Stern, 2018).

In the 1990's, major discovery was that the HPV major capsid protein L1 could self-assemble in arrays of 72 pentamers resulting in empty virus-like particles (VLPs) that present an exterior surface closely mimicking native HPV particles but without the HPV DNA genome and thus being non-pathogenic (Zhang et al., 2016; Mohsen et al., 2017). Moreover, HPV L1-based VLPs appeared highly immunogenic and able to elicit effective and protective humoral immune responses against native type-specific HPV (Mohsen et al., 2017).

Three current prophylactic VLP-based HPV vaccines for the prevention of HPV infection and related diseases have been designed and developed (Figure 4). Briefly, the Cervarix® is the first licensed HPV vaccine from GlaxoSmithKline, with a bivalent specificity directed against HPV-16 and HPV-18. The two other HPV vaccines are both from Merck & Co. Inc., the Gardasil-4® (also called Gardasil®), the second licensed vaccine, directed against HPV-6, HPV-11, HPV-16 and HPV-18; and finally the Gardasil-9® targeting the four types included in the Gardasil-4® plus five additional HPV types (HPV-31, HPV-33, HPV-45, HPV-52 and HPV-58) (Harper & DeMars 2017; Pinto et al., 2018).

Other differences between the vaccines include the concentration of each of the L1 VLPs, and the ratio of antigen to adjuvant. Gardasil-4® has two-fold higher concentrations of

HPV-16 L1 VLP and an equal concentration of HPV-18 L1 VLP compared with Cervarix®. Gardasil-9® contains twice more HPV-18 L1 VLP, 50% more HPV-16 antigen and more than twice more adjuvant level contained in Gardasil-4®. The implementation of vaccination programs based on a one-, two- or three- dose schedule (0, 1 and 6 months) of intramuscular-based immunization of young adolescent girls and boys are currently ongoing in many countries (Harper & DeMars 2017; Pinto et al., 2018).

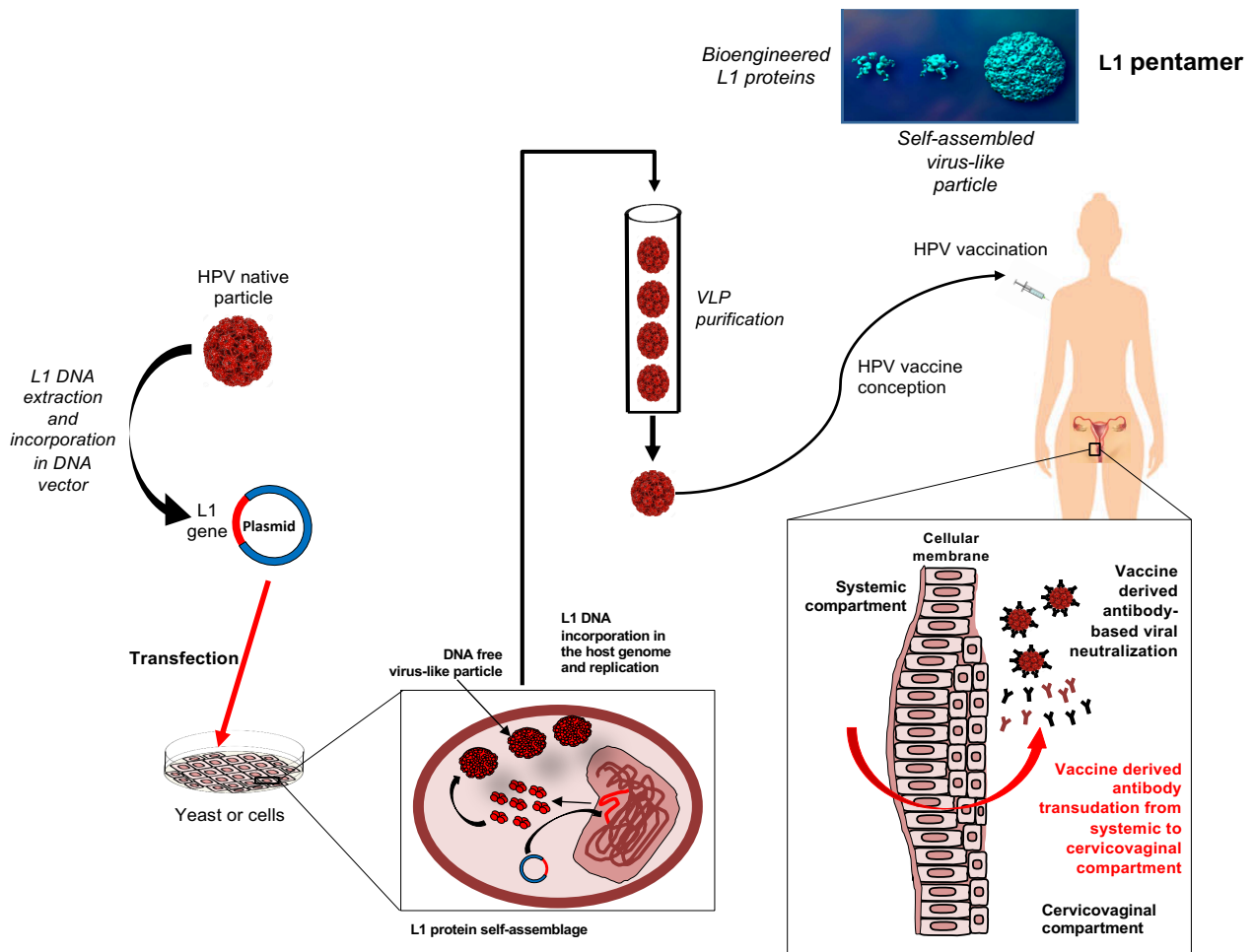


Figure 4. From native human papillomavirus (HPV) to virus-like particles (VLPs)-based HPV prophylactic vaccines. Attenuated virus vaccines have been shown to be more immunogenic than vaccines using only part of virus such as recombinant proteins or peptides. However, attenuated virus vaccines pose a risk because of the possibility of reactivation and recombination of the virus within the vaccinated individual. Vaccines composed of the conformational external structure of the native virus represent a safer alternative to overcome the risk of post-vaccination viral reactivation or recombination. This is the case of the prophylactic HPV vaccine. The viral capsid of HPV is mainly composed of the L1 protein which has the ability to self-assemble into pentameric capsomer, then into a whole viral capsid formed of 72 capsomeres. Thus, when cells (293T or yeasts) are transfected with a plasmid on which the HPV L1 gene is incorporated, they can synthesize L1 proteins which will self-assemble in these cells until they form HPV-type specific L1-viruses-like particles (VLPs). These

structures are conformationally identical to the native HPV viral capsid, except that these VLPs do not contain HPV DNA. These VLPs mimic the native viral capsid of HPV and are thus highly immunogenic. After lysis of the cells and purification of the cell lysate, a suspension of VLP is obtained. These VLPs are part of the current prophylactic vaccines. When inoculated by intramuscular injection, they induce an HPV type specific immune response corresponding to the VLP contained in the vaccine. This humoral immune response, initially systemic, is exported to the mucosal compartment, the gateway for native HPV, where it provides long-term protection against future intrusions of the virus.

11.3. Immunogenicity of HPV prophylactic vaccines

Since their elaboration and licensing, the three current prophylactic HPV vaccines have been thoroughly studied, in regard to their safety, efficacy, immunogenicity and cost-effectiveness (Harper & DeMars 2017; Pinto et al., 2018).

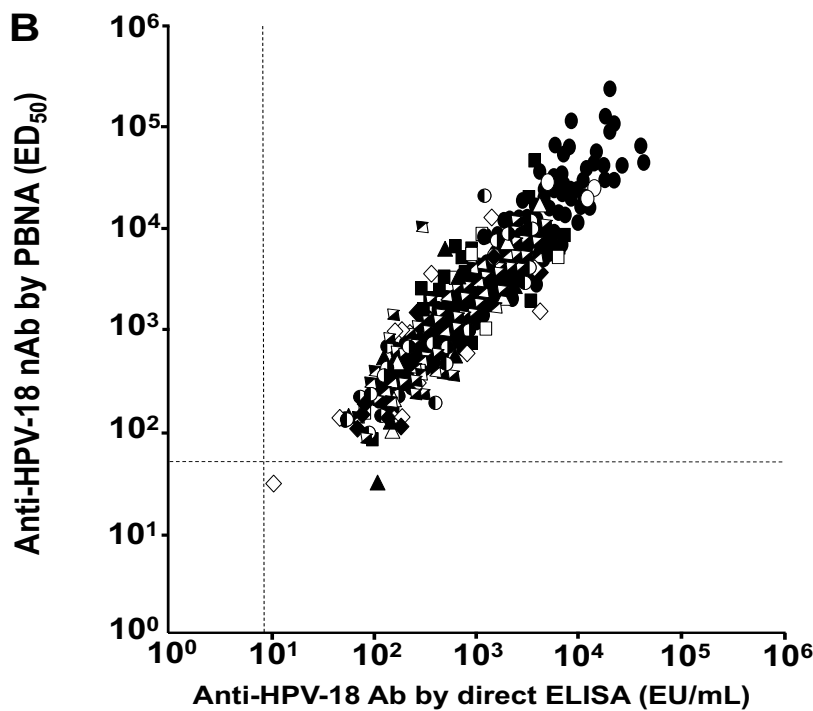
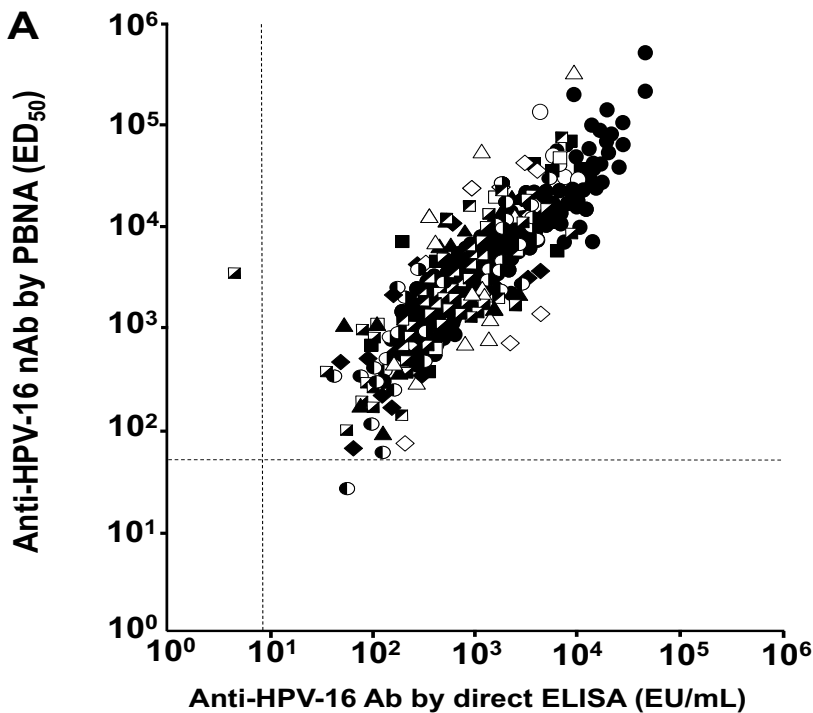
WHO guidelines for HPV vaccines have indicated that “neutralization assays are considered ‘the gold standard’ for assessing the protective potential of antibodies induced by the HPV vaccines” (WHO 2007; WHO 2008). Neutralization assays based on multiple epitopes and independent of vaccine material are considered the ‘gold standard’ for unbiased assessment of the protective potential of vaccine-induced antibodies. However, their use in large clinical trials is challenging. In addition, once the neutralizing antibody response has been well characterized, the use of alternative assay methods, such as ELISA, may be proposed as long as they are supported by a detailed analysis of the correlation with the neutralization test. Both the direct ELISA and the pseudovirions-based neutralization assay (PBNA) were used in 2008 for the initial assessment and registration of GSK’s cervical cancer vaccine, Cervarix®, worldwide, by demonstrating HPV-16/-18 antibody responses in vaccinated women enrolled in GSK’s cervical cancer vaccine trials (Dessy et al., 2008) (Figure 5).

In a recent review on the immunogenicity of the three licensed HPV prophylactic vaccines, Pinto and colleagues showed that Cervarix® and both Gardasil® vaccines are fully effective in HPV-naive young women allowing to achieve a complete seroconversion and providing nearly full protection against HPV infection in advanced clinical trials (Pinto et al., 2018). The three HPV vaccines elicit high titers of vaccine HPV-type specific serum and mucosal antibodies which persist several years after vaccination (Pinto et al., 2018).

Frazer detailed the acquisition of the vaccine induced HPV humoral response following the 3-dose schedule protocol (Frazer et al., 2010). The measurable primary immune response is characterized by a low affinity serum IgM pool, after a period of 4–8 days

following the first dose, reaching a plateau between 12 and 18 days after immunization and then declines. When the second and the third dose are administered, a further antibody response occurs, characterized by a shorter time to induction and a markedly higher level of antibody typically constituted by IgG and IgA with higher affinity for HPV antigens and persisting several years after the immunization (Frazer et al., 2010; Gonçalves et al., 2016).

In regard to these observations, it is obvious that the robust immunogenicity elicited by HPV vaccines markedly contrasts with the immune responses observed after natural infection in which seroconversion is not always the rule and the protection against subsequent HPV infection is limited to individuals with relatively high levels of naturally-acquired antibody and mostly limited to women (Beachler et al., 2016; Pinto et al., 2018). VLPs-based HPV vaccine generate strong reactions from the immune system and produce antibody titers that are much greater than those prompted by natural infection (Dawar et al., 2007).



- | | | | |
|-------|-----------|-----------|-----------|
| ● M7 | □ M25-M32 | ▲ M45-M50 | ◐ M63-M68 |
| ○ M12 | ◆ M33-M38 | △ M51-M56 | ◑ M69-M74 |
| ■ M18 | ◇ M39-M44 | ◌ M57-M62 | ◒ ≥M75 |

Figure 5. Demonstration of HPV-16/-18 neutralizing antibody responses in vaccinated women.

Relationship between antibody titers measured by direct ELISA and pseudovirions-based neutralization assay (PBNA) at each time-point in studies HPV-001 and HPV-007 for (A) HPV-16 and (B) HPV-18, demonstrating HPV-16/-18 neutralizing antibody responses in vaccinated women enrolled in GSK's cervical cancer vaccine trials, and used in 2008 for the initial registration of GSK's cervical cancer vaccine.

Ab, antibody; ED50, effective dose producing 50% response; ELISA, enzyme-linked immunosorbent assay; EU/mL, ELISA units per milliliter; M, month; nAb, neutralizing antibody. Dotted lines indicate the cut-off values for both assays. Adapted from (Dessy et al., 2008).

Given that the current HPV prophylactic vaccines are based on HPV L1 VLPs mimicking the external conformation of the capsid of native HPV, the question arises about why vaccines

induce stronger anti-HPV humoral immune responses than the antibody response induced by natural infection.

In order to provide insight in the difference between the naturally acquired HPV humoral immunity and the vaccine induced HPV antibody response, Scherpenisse and colleagues have evaluated multiple aspects of the humoral antibody response in both naturally infected and vaccinated individuals (Scherpenisse et al., 2013b). In this study, both natural infection and vaccination induced similar levels of serum IgG responses, mostly dominated by IgG1 (78% and 92%, for HPV16 and HPV18 natural infections, respectively) and IgG3 (20% and 6%, for HPV16 and HPV18 natural infections, respectively) subclasses with small amount of IgG2 and IgG4 (Scherpenisse et al., 2013b). However, vaccine-derived HPV-16 and HPV-18-specific sera were more likely to inhibit artificial infection based on homologous pseudovirions (PsV) (99.7% and 99.6%, respectively) compared to sera from naturally anti-HPV-16 and anti-HPV-18 seropositive individuals (94.5% and 83.1%, respectively). In addition, both naturally and vaccine induced antibody responses were mostly genotype specific and the cross-reactivity with additional heterologous genotypes ranged between 28% to 88% for PsV16 and between 18% to 73% for PsV18 in vaccinated sera compared with 45% to 62% for naturally seropositive individuals (Scherpenisse et al., 2013b). Finally, vaccine-derived HPV-16 and HPV-18 antibodies showed a significant 3 times higher avidity for VLP-16 and VLP-18 (86% and 84%, respectively), than naturally induced antibodies (35% and 28%, respectively). Thus, the significant difference in the avidity between naturally and vaccine induced HPV antibodies may be one of the main reasons which could explain the different level of protection elicited by natural HPV infection and vaccination (Scherpenisse et al., 2013b).

Another aspect that HPV prophylactic vaccines need to reach to be suitable for an optimal protection against HPV infection is an extended spectrum to a wide range of HPV genotypes. In the study of Scherpenisse and colleagues vaccine-derived HPV-specific antibodies were mostly genotype-specific and demonstrated mainly cross-reactivity with phylogenetically related genotypes of the same species (α -9 or HPV16-like: HPV-31, HPV-33, HPV-35, HPV-52, HPV-58; and α -7 or HPV18-like: HPV-39, HPV-45, HPV-59, HPV-68) (Scherpenisse et al., 2013b). Other studies have reported similar patterns of cross-reactivity of vaccine-induced HPV-specific antibodies dependent on the phylogenetic proximity of the viruses with Cervarix® inducing higher cross-reactivity than Gardasil-4® (Scherpenisse et al., 2013a; Barzon et al., 2014; Bissett et al., 2014; Godi et al., 2015; Leung et al., 2015; Pinto et al., 2018). Furthermore, Bissett and colleagues also demonstrated that vaccine induced cross-neutralization of non-vaccine types PsV was mediated by a minority of

antibodies with multiple specificities and recognizing single or multiple epitopes of non-vaccine types, rather than possible weak interactions of predominantly type-restricted HPV-16 antibodies with some parts of non-vaccine epitopes exhibiting some degree of similarity to HPV-16 antigens (Bissett 2014). In regards to the findings of Bissett and colleagues, further studies are needed to explore such subpopulation of non-specific but cross-reactive antibodies elicited by HPV vaccine, which may be used for the production of broader spectrum HPV vaccines.

Along with the ability to provide complete and rapid seroconversion, to induce HPV type-specific antibodies exhibiting high affinity for HPV antigens, strong avidity for native HPV particles, high rates of cross-reactivity with non-vaccine HPV types and demonstrating full protection against subsequent HPV infection, HPV prophylactic vaccines also need to elicit high titers of neutralizing HPV antibodies both in the serum, but more especially in the mucosal compartments that are the gateway for oncogenic HPV. In addition, it is crucial that the vaccine-induced strong neutralizing antibody titers persist overtime, and remain high, throughout the risk period of vaccinated individuals. Studies addressing this issue demonstrated that both Cervarix® and Gardasil® vaccines induced high titers of HPV type-specific antibodies, not only in the serum, but also in mucosal area such as the cervix and the oral cavity (Schwarz et al., 2010; Scherpenisse et al., 2013a; Nakalembe et al., 2014; Handisurya et al., 2016; Harper & DeMars, 2017; Signorelli et al., 2017; Pinto et al., 2018). For example, in a cross-sectional study assessing the immune response elicited by Cervarix® conducted in young female Ugandan teenagers (10-16 years), vaccinated young girls exhibited high levels of vaccine HPV type-specific antibodies 15 to 20 times higher than the titers detected after natural infection (Nakalembe et al., 2014). In addition, antibody levels to non-vaccine HR-HPV types (31, 33, 35, 45, 52 and 58) were all significantly higher in the vaccinated group than in the unvaccinated group ($p < 0.01$) (Nakalembe et al., 2014). However, in a study evaluating the immunogenicity of the Cervarix®, Scherpenisse and colleagues reported that although the Cervarix® elicit high titers of neutralizing IgG and IgA both in the serum and in the cervicovaginal cavity, the IgA detected in the cervicovaginal cavity did not exhibit cross-reactivity to non-vaccine HPV types (Scherpenisse et al., 2013a). Assumptions about possible active transudation or passive exudation of systemic vaccine antibodies from the serum to mucosal sites are evoked, given the strong correlation of IgG levels between serum and mucosal compartments (Schwarz et al., 2010; Li et al., 2011; Scherpenisse 2013a; Pinto et al., 2018). These data suggest that the extended protection elicited by the cross-reactivity of vaccine-induced HPV antibodies may be mostly restricted to IgG, rather than IgA antibodies.

Additional studies are thus needed to better understand the origin of mucosal antibodies after vaccination as no correlation of vaccine induced IgA levels can be evidenced between systemic and mucosal compartments (Scherpenisse et al., 2013a; Pinto et al., 2018). One hypothesis is that vaccine antigens could be captured and supported by the APC circulating in the systemic compartment and then transported to the mucosal lymphoid tissues to stimulate local B-cells and induce a local production of HPV vaccine-type specific IgA and IgG (Naz et al., 2012; Scherpenisse et al., 2013a).

Further analyses are also needed to differentiate the implication of each of systemic and locally induced HPV antibodies in the protection against subsequent HPV infections. Indeed, if vaccine-induced antibodies locally secreted play an important role in the mucosal protection against HPV infection, new strategies such as boosted mucosal immunization could be envisaged in order to enhance and to strengthen HPV protection elicited by the current high effective HPV prophylactic vaccines (Balmelli et al., 1998; Fraillery et al., 2009). Similar to Cervarix®, both quadrivalent and ninevalent Gardasil® vaccines also provide, each other, comparable antibody responses against their common targeted HPV types (HPV-6/11/16/18), but also against the five additional HPV types for the Gardasil-9® (HPV-31/33/45/52/58) (Frazer et al., 2010; Nakalembe et al., 2014; Mugo et al., 2015; Handisurya et al., 2016; Huh et al., 2017; Signorelli et al., 2017; Huang et al., 2018; Pinto et al., 2018; Ruiz-Sternberg et al., 2018). According to the assumption that high titers of vaccine-induced HPV antibodies would predict good and sustained protection, both Cervarix® and Gardasil® vaccines have good immunogenic susceptible to allow durable protection against subsequent HPV infection with related vaccine types. However, although both Gardasil® vaccines induced high titers of anti-HPV-16/-18 antibodies, these latter appear lower compared to those elicited by the Cervarix® (Barzon et al., 2014; Einstein et al., 2014; Godi et al., 2015; Leung et al., 2015; Leung et al., 2018). Indeed, studies comparing the immunogenicity elicited in young girls (9-14 years) after 12 months and 36 months by a two- or three- doses immunization schedule, between the Cervarix® and the 4-valent Gardasil®, have shown that 2 doses of the Cervarix® vaccine induced significantly higher and sustained HPV-16/-18 antibody responses compared to 2 and even to 3 doses of 4-valent Gardasil® (Leung et al., 2015; Leung et al., 2018).

Along with the amount of anti-HPV antibodies elicited by the current HPV prophylactic vaccines, ongoing clinical trials also report regularly insight on the evolution over time of antibody titers induced by Cervarix® and both Gardasil® (quadrivalent and ninevalent) vaccines. All the three vaccines induce high titers of anti-HPV antibodies in vaccinated individuals, which remain for a long time, well above the titers induced by a

natural infection (Safaeian et al., 2013; Naud et al., 2014; Godi et al., 2015; Leung et al., 2015; Vesikari et al., 2015; Harper & DeMars, 2017; Huang et al., 2018; Pinto et al., 2018; Ruiz-Sternberg et al., 2018). For example, Cervarix® has showed to induced high and durable anti-HPV-16/-18 antibody titers for nearly ten years after vaccination (Petäjä et al., 2011; Naud et al., 2014; Schwarz et al., 2017). Likewise, the quadrivalent Gardasil® vaccine has also demonstrated high and sustained immunogenic potential in inducing high titers of anti-HPV-6/11/16/18 antibodies for at least 5 years and up to 9 years after vaccination (Olsson et al., 2007; Nygård et al., 2015). In addition, the last licensed Gardasil-9® vaccine has been reported to elicit similar anti-HPV-16/-18 antibody responses as the 4-valent Gardasil®, five years after vaccination (Guevara et al., 2017; Ruiz-Sternberg et al., 2018). However, as for the titers of anti-HPV16/18 which are higher in women vaccinated with the Cervarix® than those receiving Gardasil® vaccines, it has been shown that antibody titers elicited by the Cervarix® remain stable over time, contrarily to anti-HPV-18 elicited by both 4- and 9-valent Gardasil® vaccines which decline over time until being undetectable (Olsson et al., 2007; Einstein et al., 2014; Leung et al., 2015; Guevara et al., 2017; Leung et al., 2018). Similar patterns have been also reported for anti-HPV-45 (a HPV type phylogenetically related to HPV-18) antibody titer in women vaccinated with the Gardasil-9® vaccine in whom nearly 15% of vaccinated individuals loss the seropositivity for vaccine-induced anti-HPV45 antibody (Harper & DeMars 2017; Pinto et al., 2018). The instability of Gardasil-derived anti-HPV-18 and anti-HPV-45 antibodies over time could affect the protection afforded by these vaccines.

According to these findings and in regard to the protection against the two most oncogenic HPV types (HPV-16/-18) which represent together more than 70% of cervical cancers and near to 90% of anal cancers in women and men respectively, the use of Cervarix® in vaccination programs seems to be more interesting than both the 4- and 9-valent Gardasil® vaccines. However, although Gardasil® vaccines induce lower vaccine-derived antibody titers than Cervarix®, the titers elicited by Gardasil® vaccines are still well above the antibody titers induced by natural infection, with the exception of anti-HPV-18 and anti-HPV-45 antibody titers that decline over time. Furthermore, the additional protection against the two most clinically relevant LR-HPV (HPV-6/-11) and also the additional five most detected oncogenic HPV (HPV-31/-33/-45/-52/-58) provided by Gardasil® vaccines, make these vaccines suitable for immunization programs in order to extend the protection to a larger spectrum of HPV types than the protection elicited by Cervarix®.

HPV-16 and HPV-18 cause most oropharyngeal cancers, which are increasing in incidence, particularly among males (Chaturvedi et al., 2011). The efficacy of HPV vaccines for oropharyngeal cancers has not yet been demonstrated. The demonstration of HPV-specific

antibodies after vaccination would bring biological plausibility of vaccine protection, since HPV antibodies are believed to be the main effectors of protection and efficacy against HPV infection at various mucosal sites. Oral samples constitute a useful resource for the detection of HPV-16 and HPV-18-specific antibodies in saliva following HPV vaccination (Parker et al., 2016; Pinto et al., 2018). Thus, vaccination of males with Gardasil® vaccine induces detectable HPV-specific antibody levels at the oral cavity that strongly correlate with circulating levels (Pinto et al., 2016; Parker et al., 2019). Reduced detectability of oral and serum HPV-16 and HPV-18 antibodies was however observed at months 18 and 30 after initiation of the prophylactic quadrivalent HPV vaccination (Parker et al., 2019). It is still uncertain whether the vaccine-induced antibody levels at the oral cavity are sufficient to provide long-term protection against acquisition of oral HPV-16 and HPV-18 infections.

11.4. Effectiveness of HPV prophylactic vaccines

Cervarix® has been shown to induce cross-reactivity in healthy girls/women with a vaccine effectiveness of 34.5% against persistent HPV-6/-11 at 48 months (Szarewski et al., 2013), and also associated with a modest reduction in the incidence of genital warts in young girls (Howell-Jones et al., 2013).

Cervarix® and Gardasil® vaccines have been shown in randomized control trials to be highly efficacious against caused high grade cervical intra epithelial disease (CIN2/3) caused by HPV-16/-18 in 15-26 years old women naïve for these HPV types at trial entry and during the 3 shot immunization schedule (0, 1 or 2 and 6 months) (Kjaer et al., 2009; Paavonen et al., 2009). The Cervarix® bivalent vaccine was evaluated in a phase III study sponsored by GlaxoSmithKline called the Papilloma Trial Against Cancer in Young Adults (Paavonen et al., 2009; Lockett and Feldman, 2016; St Laurent et al., 2018). In this multinational prospective double-blind placebo-controlled trial, the vaccine was 92.9% effective in preventing CIN 2 in 18,000 women ages 15–25 with either normal cervical cytology or low-grade cervical dysplasia. The vaccine was 52.8% effective in preventing these lesions as well as 33.6% effective in preventing high risk CIN 3 lesions in patients with a history of HPV infection.

Additional trial endpoints were evaluated for the quadrivalent vaccine and high (>96%) efficacy was shown for HPV-6/-11/-16/-18 related vulvar and vaginal intra epithelial neoplasia and external genital warts (Dillner et al., 2010). The quadrivalent Gardasil® HPV vaccine was also evaluated in a series of Merck & Co. Inc. sponsored trials: Females United to Unilaterally Reduce Endo/Ectocervical Disease (FUTURE) trials (Garland et al., 2007). In

the first smaller trial, quadrivalent HPV vaccine was 100% effective against CIN 2/3 in HPV naive women. The follow-up trial, FUTURE II, was a larger phase III double-blinded, multinational prospective, placebo-controlled trial of over 12,000 women. Qualified study subjects were between the ages of 15–26 without a history of abnormal Papanicolaou smears and had less than four lifetime sexual partners. In this group, quadrivalent HPV vaccine was 98% efficacious against a composite of CIN 2/3, HPV-16/-18 infection, as well as adenocarcinoma in situ in women aged 15 to 26. Additionally, the vaccine was 42% effective against HPV-16 and 79% effective against HPV-18. In the same group, quadrivalent HPV vaccine was, respectively, 57 and 45% effective against HPV-16- and HPV-18- associated CIN 2/3, preventing approximately 17% of all CIN 2 lesions. Importantly, the quadrivalent HPV vaccine demonstrated limited cross-protection, 32.5%, against CIN 2/3 and AIS in the FUTURE trials.

In randomized clinical trials in 16-23 years-old men, the quadrivalent vaccine has been shown to achieve >90% efficacy against HPV-6/-11/-16/-18 caused external genital warts in heterosexual men and > 73% efficacy against anal intra-epithelial neoplasia in homosexual men (Stanley, 2010). A sub-population of MSM was analyzed and showed an efficacy of 77.5% (95% CI: 39.6 to 93.3) against both HPV type 6-, 11-, 16- and 18-related anal intraepithelial neoplasia (Palefsky et al., 2011).

The efficacy of the nonavalent Gardasil® HPV vaccine was evaluated in a phase IIb-III double-blinded, randomized international non-inferiority trial supported by Merck & Co. Inc. (Lockett and Feldman, 2016; St Laurent et al., 2018). In 14,215 low risk women ages 16–26 randomized to a 3-dose regimen of either nonavalent HPV vaccine or quadrivalent HPV, the nonavalent HPV vaccine was 96% effective in preventing high-grade CIN, as adenocarcinoma in situ, high-grade VAIN, vulvar cancer, and vaginal cancer associated with the nine HPV subtypes targeted by the nonavalent HPV vaccine. Nonavalent HPV vaccine was 96% effective in preventing persistent infections related to the same HPV-types. However, analysis of women including those with prior HPV infection showed no difference in cervical, vaginal, or vulvar disease between the two vaccines (Lockett and Feldman, 2016).

There is finally evidence for population effectiveness for the quadrivalent vaccine (Wang et al., 2018). In 2008 Australia offered a universal publicly funded vaccination program with the quadrivalent vaccine to all women aged 12-26 years and achieved, overall, 60-70% coverage. Audit of new female patients with external genital warts, 28 years or less attending a large sexual health clinic in Melbourne, Australia before and after vaccine introduction showed a fall in incidence of external genital warts after vaccine introduction in this age group of up to 40% women older than 28 years who were not in the group eligible for

free vaccines showed no fall in incidence (Fairley et al., 2009). National surveillance for genital warts conducted following this initiative demonstrated also a 28% drop in diagnosis rates of genital warts in heterosexual men. There was, however, no decrease in genital warts observed in MSM. These data suggest that the vaccination of women may prevent transmission of HPV vaccine types to men (Donovan et al., 2011). There are currently no studies that have directly demonstrated that HPV vaccination of men will prevent transmission of vaccine types to women. However, mathematical models predict that the addition of a routine male HPV program would prevent additional cases of genital warts and cervical cancer among women, based on assumptions about transmission of HPV from men to women (WHO 2017).

Although the evidence base to support the immunogenicity of HPV vaccines in HIV-infected persons is reasonable, the evidence base to support the efficacy of HPV vaccines in HIV-positive individuals remains currently inconsistent (Lacey et al., 2019).

12. Conclusion

Finally, despite the viral mechanisms put in place by HPV to escape the host immune system, a specific humoral response against HPV is still measurable in a large number of individuals following the infection. This naturally acquired humoral immune response appears to be protective against subsequent infections only in some cases and it seems to be not persistent. This suggests that most unvaccinated people exposed to HPV, although having developed a humoral response, are still at risk for future HPV infections. Unlike seroconversion for L1 derived antibody response, which is not always the rule after exposure to the virus, antibodies to certain early viral proteins such as the E6 and E7 proteins seem to be correlated to early stages of head and neck cancers and could represent potential markers for the early diagnosis of these cancers. In contrast to the naturally acquired humoral immune response, the current prophylactic vaccines have been shown to be highly immunogenic, inducing very high titers of persistent and specific antibody with the induction of memory B-cells able to produce specific antibodies, almost 10 years after vaccination. Vaccine-induced humoral immunity provides a protection against a large spectrum of the HPV types the most detected in cancers or HPV-related diseases. Since several different serological assays are currently used to evaluate the humoral immunity induced by HPV infection or vaccination, a standardization of cut-offs and thresholds of positivity and significance, becomes necessary in order to improve and facilitate the comparison of the data generated by these serological assays and to harmonize the conclusions.

13. Expert commentary

It is obvious that natural infection with HPV induces a modest humoral immune response which seems to confer a certain degree of protection to subsequent HPV infection, mostly to homologous HPV types and particularly restricted to women (Pinto et al., 2018). However, a large part of the exposed population remains seronegative and those who develop seropositivity become seronegative with time, suggesting that the modest protection afforded by the naturally acquired humoral immunity, would not always persist longtime after infection (Mooij et al., 2014a; Beachler et al., 2016; Pinto et al., 2018). This indicates that most unvaccinated people exposed to HPV, although having developed a humoral immune response, are still at risk for future HPV infections.

In contrast to the naturally acquired humoral immune response, the current prophylactic vaccines have been shown to be highly immunogenic, inducing very high titers of persistent, type-specific and also cross-reactive antibodies with the induction of memory B-cells able to produce specific antibodies, almost 10 years after vaccination (Beachler et al., 2016; Pinto et al., 2018). Vaccine-induced humoral immunity provides a protection against a large spectrum of the HPV types the most detected in cancers or HPV-related diseases. These vaccines show a particularly high immunogenicity and the highest protective efficacy in young (9 to 14 years old) female and male, previously uninfected (no HPV DNA) and seronegative for anti-HPV antibodies (Arbyn et al., 2018; Pinto et al., 2018). Thus, most of national health policies around the world recommend vaccination mostly for young girls, but also for young boys (9 to 14 years old) with possible catch-up for HPV DNA negative young women (up to 18-19 years old) and also for high-risk young adult MSM (up to 26 years old) in first instance, irrespective of HPV molecular test (Arbyn et al., 2018; Pinto et al., 2018).

In this context, it is a large part of the population at risk for HPV-related cancers (i.e. healthy women older than 18 years old and people already positive for HPV DNA but seronegative for anti-HPV or those negative for HPV DNA but seropositive for anti-HPV) that cannot take advantage of the herd immunity afforded by HPV vaccination and who are therefore relegated to alternative prevention measures (molecular and cytological screening for HPV DNA and precancerous lesions). Although the vaccination effectiveness is much more evident in young healthy girls and boys, there are some evidences that a protection against HPV infection, similar to that seen in vaccinated young adolescents is also developed in vaccinated women over 25 years old (Skinner et al., 2014; Schwarz et al., 2015; Schwarz et al., 2017; Harper & DeMars, 2017). In addition, among women already seropositive for a

HPV type included in a vaccine, but HPV DNA negative at the time of vaccination, HPV vaccines have shown to induce high antibody titers similar to that observed in healthy young adolescents (Villa et al., 2006; Olsson et al., 2009; Paavonen et al., 2009; Bhatla et al., 2010; Harper & DeMars, 2017). These results indicate that the age of vaccination should not be limited to young adolescents, but could be extended to adults well beyond 25 years of age. In addition, individuals who already have HPV exposure markers should not be excluded from the targeted population of vaccination. The inclusion of these two populations at-risk for HPV-related cancers in HPV vaccination recommendations could broaden the herd immunity elicited by HPV vaccination.

Finally, other alternatives to improve or strengthen HPV vaccination are actively studied. Two of these alternatives are the possibility of developing a pan-HPV vaccine covering a full spectrum of HPV types (Tumban et al., 2011; Tyler et al., 2014a; Tyler et al., 2014b; Tyler et al., 2014c; Boxus et al., 2016) and the necessity to improve the vaginal and anal humoral response using a boosted mucosal vaccine and other modes of administration (Frailery et al., 2009; Hunter et al., 2011; McKay et al., 2017; Singh et al., 2018).

14. Five-years view

The majority of vaccination programs against HPV infection implemented throughout the world and based on the current prophylactic vaccines are focused on a 3-dose schedule in a 6-months period in order to strongly stimulate the immune response and induce high antibody titers in vaccinated individuals (Harper & DeMars 2017; Pinto et al., 2018; Ruiz-Sternberg et al., 2018). However, in most parts of the world where HPV infection and associated cancers are among the major public health concerns, people do not always have easy access to health facilities where HPV vaccine are administered as the primary prevention option. Thus, the current programs of three doses over a period of 6 months seem quite restrictive for these areas (D'Addario et al., 2017). A primo-stimulation with one dose followed by a booster, or a single dose could be an alternative to retain and to cover quite easily the target populations in these areas with limited resources (Harper & DeMars 2017; Pinto et al., 2018). Reduced dose schedules have demonstrated non-inferior antibody responses in clinical trials in young girls (Leung et al., 2015; D'Addario et al., 2017; Leung et al., 2018; Pinto et al., 2018). These evidence of good immunogenicity of a 2-doses schedules have led to the approval and the recommendation of a 2-doses schedule with Gardasil-9® for young girls and boys (9 to 14 years old) in the USA (Meites et al., 2016). But as for the 3-doses schedules, the two-doses schedules do not reduce the 6 month-immunization period and could be a risk for losing sight

of adolescents who received the first dose, especially in countries with limited access to healthcare facilities (D'Addario et al., 2017). In order to optimize immunization coverage and boosting the herd immunity against HPV infection, it becomes necessary to reduce the vaccination schedule to a single dose. To date, immunogenicity data on single dose vaccine are limited and clinical trials are currently underway. Nevertheless, preliminary data suggest that a one-dose schedule of HPV vaccine would be sufficient to induce considerable antibody titer able to confer protective immunity against HPV (Safaeian et al., 2013; Sankaranarayanan et al., 2016; Harper & DeMars 2017; Pinto et al., 2018). With the current ongoing clinical trials, the next five years will provide a more concrete insight on the immunogenicity of a single dose.

Finally, although the various clinical trials currently in progress throughout the world show that current prophylactic vaccines theoretically confer protection against HPV infection and associated cancers, the global impact of the wide vaccination on the incidence of these cancers will only be felt in the next twenty to thirty years, period during which the currently vaccinated girls and boys will reach their critical age for HPV-associated cancers.

15. Key issues

- Naturally acquired HPV-type specific antibodies provide modest protection against subsequent infections for women, but not for men.
- Several men and women exhibiting humoral immunity following natural HPV infection are still, nevertheless, at risk for subsequent or recurrent HPV infections and associated cancers.
- The impact of HIV on the protective effect conferred by the naturally acquired HPV-type specific antibodies is still unclear.
- The current approved three prophylactic vaccines, Cervarix®, Gardasil-4® and Gardasil-9® are all strongly immunogenic and induced high and persistent titers of anti-HPV antibodies highly type-specific and also cross-reactive, both in the serum and in the mucosal compartments. Thus, this vaccine-induced humoral response confers a strong and durable protection against subsequent and recurrent HPV infections in young girls and boys.

- However, some concerns arise about the vaccination with these three vaccines. Firstly, the decline of vaccine-induced antibody to HPV18 and related phylogenetic types suggest a possible progressive loss of the protection elicited against these HPV, after vaccination with both the two Gardasil® vaccines. On the other hand, there is an urgent necessity to reduce the dose schedule from a 6-months 3-doses schedule to a unique but highly immunogenic and protective dose. The extension of the age of vaccination should be also thoroughly studied for a possible extension from the current recommendation (9-14 years old) to a possible recommendation of age vaccination well beyond the age of 25 years to 35/40 years old, both for adult women and men which are the most at-risk age group for HPV-related cancers. Along with a reduced dose schedule, a vaccination program including young adult individuals (25-40 years old) and also individuals with pre-existing HPV infection markers (HPV DNA positivity and/or anti-HPV antibodies seropositivity) would be a great alternative for extend the vaccine coverage. Particularly in countries with limited resources which are also both the countries where the current vaccination recommendations are difficult to be implemented and also those with the highest prevalence and incidence of HPV infection and related cancers. These alternatives could help to broaden the herd immunity against HPV in the most at-risk population living in the most at-risk areas.

IV. Problématique et objectifs de l'étude

Le cancer anogénitaux associés aux Papillomavirus humains à haut-risque oncogène (HR-HPV), en particulier le cancer du col de l'utérus, constituent des défis majeurs de santé publique sur le plan mondial en général, et particulièrement en Afrique subsaharienne où le cancer du col de l'utérus devient progressivement l'un des premiers cancers de la femme (Mboumba Bouassa et al., 2017). Bien que ce cancer soit évitable, notamment avec les stratégies de prévention primaire basées sur la vaccination des jeunes filles (9 à 14 ans) et la prévention secondaire basée sur le dépistage moléculaire et cytologique de l'infection à HR-HPV et des lésions précancéreuses, l'incidence de ce cancer ne cesse d'augmenter chaque année (Mboumba Bouassa et al., 2017). Les stratégies de prévention qui ont fait preuve d'efficacité dans les pays développés, en réduisant drastiquement la courbe d'incidence de nouvelles infections et des cancers, sont difficilement adaptables en l'état actuel, aux pays en voie de développement (Mboumba Bouassa et al., 2017).

En effet, bien que les pays en voie de développement enregistrent les plus fortes prévalences de l'infection à HR-HPV, et particulièrement au sein des femmes adultes, certaines femmes, préalablement non-infectées, pourraient bénéficier de la vaccination de rattrapage, et ce, malgré leur âge avancé. Afin d'identifier ces femmes potentiellement éligibles pour une vaccination de rattrapage, un triage par des tests moléculaires de détection des HR-HPV devra obligatoirement être effectué. Cependant, le contexte de plusieurs pays en voie de développement, en particulier les pays d'Afrique subsaharienne, rend cette initiative difficilement réalisable. Les tests moléculaires de détection de l'infection à HR-HPV sont réalisés dans des structures de santé sophistiquées, localisées, pour la plupart des pays, dans les agglomérations principales, difficiles d'accès pour les femmes vivant dans des zones rurales reculées (Mboumba Bouassa et al., 2017). De plus, les femmes africaines immigrées vivant dans les pays développés tel que la France, sont très souvent confrontées à des situations sanitaires aussi précaires que celles qui prévalaient dans leur pays d'origine (Vigner et al., 2018a; Vigner et al., 2018b). A l'instar de celles vivant en Afrique subsaharienne, les femmes immigrées africaines vivant en France métropolitaine n'ont pas toujours accès aux structures de santé et constituent de fait un groupe très à risque pour les infections sexuellement transmissibles telles que l'infection à VIH et l'infection à HR-HPV. Aussi, il devient urgent d'adapter les stratégies de prévention actuelles afin de cibler cette catégorie de personnes à risque pour les cancers associés aux HR-HPV.

Objectifs :

- Décrire l'épidémiologie moléculaire de l'infection à HPV au sein de cohortes d'individus africains inclus et de prédire au sein de celles-ci l'efficacité de la vaccination prophylactique multivalente contre les HPV :
 - Femmes adultes non-vaccinées, vivant à N'Djamena, au Tchad, et n'ayant jamais subi de dépistage moléculaire et cytologique de l'infection à HPV et des lésions précancéreuses ;
 - Femmes adultes africaines non-vaccinées, immigrées de première génération, vivant en France et n'ayant jamais subi de dépistage moléculaire et cytologique de l'infection à HPV et des lésions précancéreuses ;
 - Homosexuels masculins vivant en République Centrafricaine.
- Appliquer les techniques immunologiques acquises ("VLP-based ELISA") pour évaluer la réponse immunitaire à IgG systémique et muqueuse contre les HR-HPV des groupes $\alpha 7/\alpha 9$, dans le but de poser les bases immunologiques de la possibilité de vaccination de rattrapage par les vaccins prophylactiques contre les HPV oncogènes au sein des femmes africaines immigrées de 1^{ère} génération vivant en France, qui sont à risque particulièrement élevé de cancer du col de l'utérus ; mais également au sein des femmes africaines vivant dans les pays en voie de développement.
- Evaluer un dispositif d'auto-prélèvement de sécrétions cervicovaginales en contexte de pays à ressources très limitées, pour l'adaptation des stratégies de dépistage actuelles aux populations isolées telles que les femmes africaines vivant dans les zones rurales, dans les pays en voie de développement, mais également les femmes immigrées africaines vivant en France métropolitaine.

Partie 2

Mémoire expérimental

I. Matériel et Méthodes

1. Conception de l'étude.

Cette étude s'articule principalement sur deux axes de recherche.

- ❖ Un axe d'épidémiologie moléculaire descriptive visant à caractériser les profils épidémiologiques de l'infection à HPV, identifier les principaux facteurs de risques associés, décrire la distribution des principaux génotypes à haut-risque circulant dans les populations africaines à risque vivant en Afrique sub-saharienne, mais également au sein de populations immigrées de première génération originaires d'Afrique sub-Saharienne et vivant en France.

- ❖ Le second volet de cette étude consiste en la caractérisation immunologique de la réponse immunitaire humorale acquise après une infection à HR-HPV chez des femmes immigrées Africaines adultes, de première génération, vivant en France et majoritairement originaires d'Afrique Centrale.

2. Population d'étude et sites d'inclusion.

Cette étude regroupe des sujets issus de trois protocoles de recherche visant à étudier l'infection à HPV et dont les inclusions ont été réalisées dans trois pays : le Tchad, pour le protocole GYNAUTO (Figure 5), la France pour le protocole ImmiPap ANRS 2019 (Figure 6) et la République Centrafricaine pour le protocole HomoPap (Figure 7). Ces trois protocoles de recherche ont chacun été approuvés par les comités d'éthique ayant autorité dans les sites d'inclusion des trois pays concernés.

a. Protocole GYNAUTO TCHAD

Afin de renforcer la couverture des programmes de dépistage dans les pays à ressources limitées et palier l'inaccessibilité des populations à risque, l'auto-prélèvement d'échantillons cervicovaginaux pour les tests moléculaires a récemment été suggéré comme une alternative très prometteuse et est de ce fait largement évalué dans les milieux à ressources limitées (Petignat et al., 2007; Alidjinou et al., 2013; Mahomed et al., 2014; Tamalet et al., 2016; Campos et al., 2017; Modibbo et al.; 2017; Mbatha et al., 2017). En effet, plusieurs études révèlent que la détection moléculaire des HR-HPV réalisée à partir d'échantillons cervicovaginaux auto-prélevés par des patientes (à domicile ou au centre de

santé) est aussi sensible que le dépistage moléculaire réalisé à partir de prélèvements collectés par un clinicien dans une structure de santé spécialisée (Alidjinou et al., 2013 ; Porras et al., 2014; Adamson et al., 2015). De plus, l'auto-prélèvement est peu coûteux, ne nécessitant pas de qualification médicale spécialisée. L'auto-prélèvement est susceptible d'être mise en œuvre individuellement par la patiente à son domicile (Haguenoer et al., 2014). Le fait qu'une femme sans qualification médicale spéciale et sans assistance particulière peut s'auto-prélever facilite son acceptabilité auprès des femmes qui peuvent ainsi préserver leur intimité (Gravitt et al., 2011; Mahomed et al., 2014). Cela facilite également l'accessibilité des populations éloignées des principales grandes structures de santé où sont conventionnellement réalisés les prélèvements génitaux à des fins diagnostic (Gravitt et al., 2011; Racey et al., 2013). Des études récentes conduites sur plusieurs sites en Afrique sub-Saharienne montrent que l'auto-prélèvement génital pour le dépistage moléculaire des infections à HR-HPV est bien accepté par les femmes, qu'il offre une performance similaire (voire même meilleure) à celle des prélèvements réalisés par un clinicien et qu'il est facilement réalisable en contexte Africain (Alidjinou et al., 2013; Mahomed et al., 2014 ; Adamson et al., 2015; Tamalet et al., 2016; Modibbo et al; 2017; Campos et al., 2017).

De ce fait, l'objectif principal du projet de recherche opérationnelle GYNAUTO visait, de façon transversale, à valider chez la femme africaine en activité sexuelle la praticabilité et l'acceptabilité du dispositif V-Veil-Up Gyn (V-Veil-Up PHARMA Ltd, Nicosie, Chypre) en tant que dispositif d'auto-prélèvement génital pouvant être utilisé pour diagnostiquer les infections à HPV. C'étude permettrait également d'obtenir des données objectives inédites sur l'importance et l'épidémiologie moléculaire des infections génitales féminines à HPV, ainsi que sur la prévalence des principales comorbidités infectieuses que constituent l'infection à VIH, et les infections à virus de l'hépatite B (VHB) et de l'hépatite C (VHC).

Cinq (5) des dix principaux arrondissements de N'Djamena ont été sélectionnés de façon aléatoire. Des éducateurs autochtones, intervenant dans les églises communautaires et les réseaux associatifs des différents arrondissements de la capitale ont contacté et invité des femmes à participer à l'étude et à se rendre au site d'inclusion, la clinique pour la santé sexuelle des femmes "*La Renaissance Plus*", où leurs données sociodémographique et comportementales ont été collectées.

- **Critères d'inclusion :**

- Femmes se rendant à la clinique « La Renaissance Plus » pour consultation
- Agées de 25 à 65 ans
- En activité sexuelle
- Ayant signé le formulaire du consentement éclairé.

- **Critères d'exclusion :**
 - Refus de participer à l'étude
 - Age < 25 ans et > 65 ans
 - Femmes ayant de la fièvre ou des symptômes de type grippal
 - Femmes ayant un antécédent de syndrome du choc toxique
 - Femmes ayant une allergie aux matières plastiques et ses dérivés
 - Femmes ayant une allergie au coton.

- **Réception des participantes et recueil des données socio-épidémiologiques et des échantillons biologiques:**

Les femmes éligibles pour l'étude et volontaires étaient reçues par un personnel médical (infirmière de préférence) qui leur expliquait et leur faisait signer la fiche de consentement éclairé. Un questionnaire était ensuite proposé aux participantes par l'infirmière afin de recueillir les données cliniques et sociodémographiques. Il était proposé aux participantes de réaliser un test de diagnostic gratuit pour le dépistage des infections à VIH, VHB et VHC. Les participantes étaient ensuite soumises aux deux méthodes de prélèvements des sécrétions cervicovaginales pour le diagnostic de l'infection à HR-HPV.

- **Prélèvements cervicovaginaux standards réalisés par l'infirmière ou le médecin :
Méthode 1 (Figure 5).**

Après réalisation du questionnaire pour le recueil des données sociodémographiques des patientes, l'infirmière effectuait le prélèvement cervicovaginal à l'aide du dispositif de prélèvement cervical ThinPrep (Hologic Inc., Bedford, Massachusetts, Etats-Unis). Brièvement, après avoir placé le spéculum (sans lubrifiant), l'infirmière utilisait le dispositif de prélèvement de type balai pour réaliser le prélèvement cervical en l'introduisant dans le conduit cervical et en effectuant 5 rotations ; le balai de prélèvement était également appliqué sur les parois vaginales avant d'être retiré et immédiatement placé dans le tube collecteur ThinPrep contenant une solution de PreservCyt (Hologic Inc., Bedford, Massachusetts, Etats-Unis) permettant de déloger les cellules épithéliales cervicales. Le balai de collecte était d'abord agité dans la solution PreservCyt afin de déloger les cellules épithéliales avant d'être éliminée [Adamson et al., 2015]. Le tube collecteur ThinPrep contenant le prélèvement réalisé par l'infirmière était conservé dans un congélateur à -20°C (ou -80°C) avant d'être transporté dans de la carboglace en maintenant la chaîne du froid, au laboratoire de virologie de l'hôpital Européen Georges Pompidou de Paris pour les analyses biologiques spécialisées.

- **Auto-prélèvement réalisé à l'aide du dispositif intra-vaginal V-Veil-Up Gyn :
Méthode 2 (Figure 5).**

Après la réalisation du prélèvement cervicovaginal standard par l'infirmière, la participante recevait une instruction pendant 15 minutes sur comment réaliser le dispositif V-Veil-Up Gyn pour réaliser un auto-prélèvement vaginal. Après avoir instruit la participante, l'infirmière quittait la salle de prélèvement et y laissait la participante réaliser, seule, son propre auto-prélèvement, sans aucune aide de la part de l'infirmière.

Brièvement, la participante devait insérer le dispositif V-Veil-Up Gyn dans son vagin ; le laisser en place durant 3 heures ; puis le retirer, le placer dans un tube collecteur Sarstedt (Sarstedt Ag & Co, Nümbrecht, Allemagne) de 15 ml et compléter le volume avec une solution tampon phosphate (PBS) pour éviter le dessèchement du prélèvement et le retourner à l'infirmière. Lorsque l'infirmière recevait le tube contenant le dispositif V-Veil-Up Gyn, elle devait vérifier que le tampon était totalement immergé dans la solution de conservation, que le tube était correctement fermé et que l'identification sur l'étiquette correspondait bien à la participante. Le tube collecteur Sarstedt contenant le prélèvement réalisé par la patiente était ensuite conservé dans un congélateur à -20°C (ou -80°C) avant d'être transporté dans de la carboglace en maintenant la chaîne du froid, au laboratoire de virologie de l'hôpital Européen Georges Pompidou de Paris pour les analyses biologiques spécialisées.

Après la réalisation des deux types de prélèvements, le questionnaire d'acceptabilité et de satisfaction au décours de l'utilisation du dispositif V-Veil-Up Gyn était ensuite proposé aux participantes.

Protocole GYNAUTO

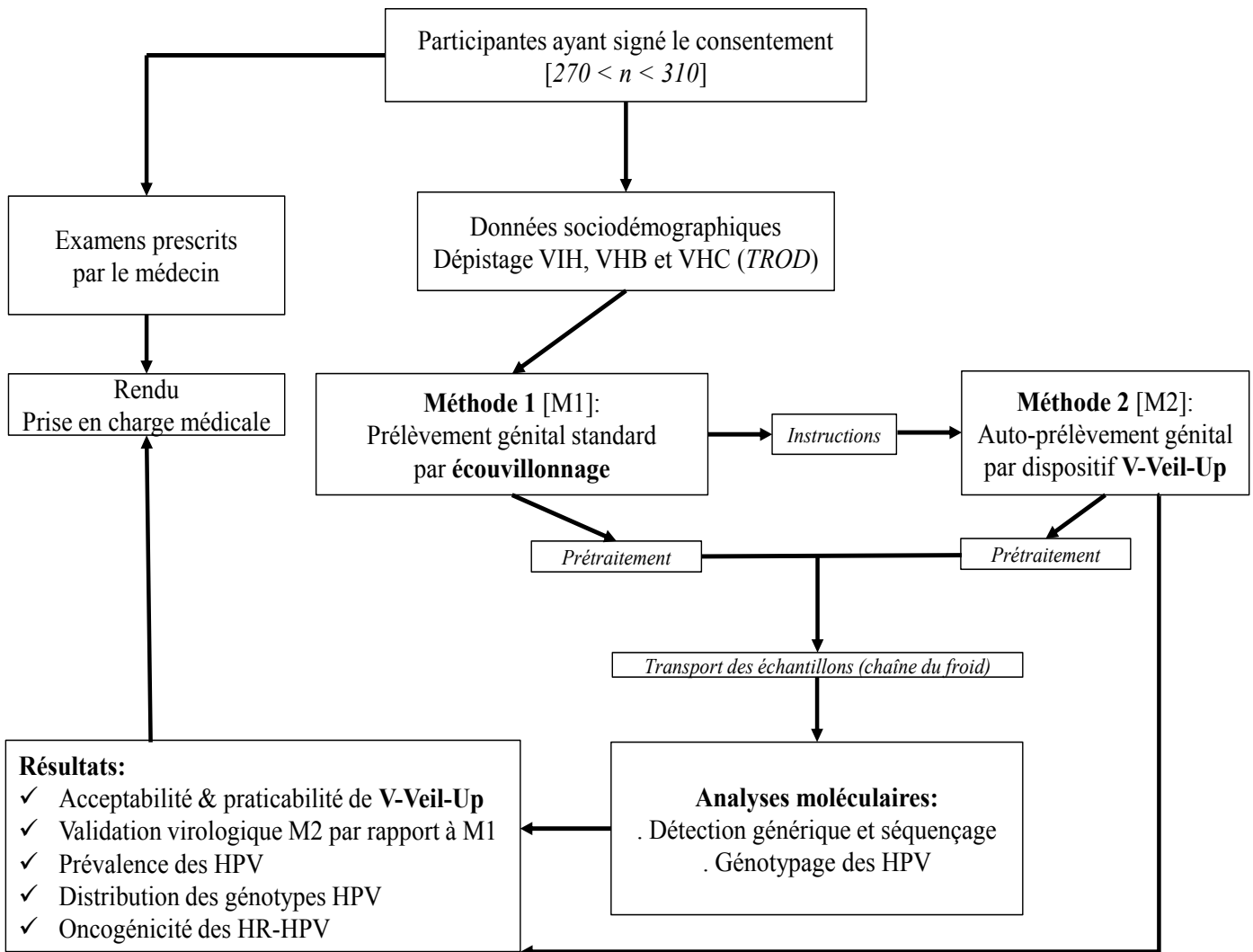


Figure 5. Diagramme directionnel de la réalisation du protocole de recherche GYNAUTO

b. Protocole IMMIPAP ANRS

Dans la population générale française, on estime qu'environ 14% des femmes ayant un frottis normal sont infectées par un génotype de HPV à haut risque et 75,6% des cancers invasifs du col de l'utérus sont attribués aux HPV-16 et HPV-18. Toutefois, cela ne prend pas toujours en compte l'origine des femmes. Notre hypothèse est que les femmes immigrées d'Afrique sub-saharienne de 2ème ou 3ème génération pourraient présenter des profils épidémiologique et immunologique (réponse humorale anti-HPV) similaires à ceux de la population générale en France, tandis que les immigrées africaines de 1ère génération (adultes ayant débuté pour la plupart leur vie sexuelle dans leur pays d'origine) présenteraient au contraire des profils épidémiologique et immunologique différents de ceux décrits dans la population générale française et reflétant plutôt l'épidémiologie moléculaire de l'infection à HPV dans la population générale en Afrique sub-saharienne (24%), tant en termes de prévalence de l'infection à HPV dans cette population spécifique qu'en termes de distribution de génotypes de HPV et également en termes de réponse immunitaire naturelle acquise après exposition naturelle.

De ce fait, l'objectif principal du projet de recherche opérationnelle IMMIPAP ANRS 2019 (toujours en cours) vise à décrire, de façon prospective, l'épidémiologie moléculaire de l'infection à HPV, la distribution des principaux génotypes de HPV d'intérêt et les facteurs de risque associés, au sein d'une population à risque, de femmes immigrées infectées ou pas par le VIH, originaires majoritairement de pays d'Afrique Centrale et vivant en France. Cette étude permettra également d'établir les profils de réponses immunitaires humorales muqueuses et systémiques acquises après une infection à HPV chez ces femmes. Enfin, elle permettra également d'identifier par la technique de Capture-HPV des signatures moléculaires d'intégration virale chez des femmes présentant des lésions précancéreuses et des cancers avérés.

- **Critères d'inclusion :**

- Femmes suivies dans les différents services des sites hospitaliers impliqués dans le protocole IMMIPAP ANRS 2019
- Originaires d'Afrique sub-Saharienne
- Agées d'au moins 25 ans, âge à partir duquel il est recommandé aux femmes d'effectuer l'examen du frottis cervical en France (HAS 2010)
- En activité génitale
- Ayant signé le formulaire du consentement éclairé.

- **Critères d'exclusion :**

- Refus de participer à l'étude

- Impossibilité d'appliquer la consigne de restriction de l'activité sexuelle 3 jours avant les prélèvements. En effet, des traces de sperme dans les prélèvements suggèreraient également la possible présence de virus, en l'occurrence des HPV, provenant ponctuellement du partenaire sexuel et pouvant n'être que transitoire dans le canal génital sans pour autant engendrer une infection persistante. Cette présence de HPV originaire du partenaire pourrait alors biaiser la pertinence des résultats.

- **Prélèvements et prétraitement :**

Pour chaque femme incluse dans l'étude, les prélèvements suivants ont été réalisés.

- Prélèvement de sang veineux (tube EDTA de 5 ml)
- Prélèvements génitaux :
 - ✓ 1 écouvillonnage de la muqueuse vaginale et de l'endocol
 - ✓ 1 lavage cervicovaginal standardisé collecté avec 3 mL de tampon PBS (phosphate-buffered saline) (Thermo Fisher Scientific, Waltham, MA, Etats-Unis) tel que décrit précédemment (Bélec et al., 1995).

Les prélèvements destinés aux analyses du programme de recherche sont traités de façon protocolaire au laboratoire de biologie médicale des différents sites d'inclusion, puis congelés (-80°C), et enfin transportés en respectant la chaîne du froid au laboratoire de virologie de l'hôpital Européen Georges Pompidou.

- **Prétraitement des prélèvements dans les sites d'inclusion :**

- Prélèvement sanguin (sang total + EDTA)
 - ✓ Séparation des constituants du sang (centrifugation 2500 trs/min pendant 10 minutes)
 - ✓ Réalisation d'aliquotes dans des tubes à hémolyse de la totalité du plasma, et congélation de l'aliquote à -80°C ;
- Ecouvillon de l'endocervix : Congélation à -80°C ;
- Lavage vaginal :
 - ✓ Centrifugation réfrigérée (+4°C) à 2000 trs/min pendant 10 minutes
 - ✓ Transfert de la fraction acellulaire dans un tube à hémolyse, puis addition de la solution de protection et conservation des ARN pour des analyses ultérieures (RNAprotect Cell Reagent de Qiagen)
 - ✓ Congélation de l'aliquote à -80°C
 - ✓ Congélation du tube primaire avec le culot de sécrétions cervicovaginales -80°C.

Protocole IMMIPAP

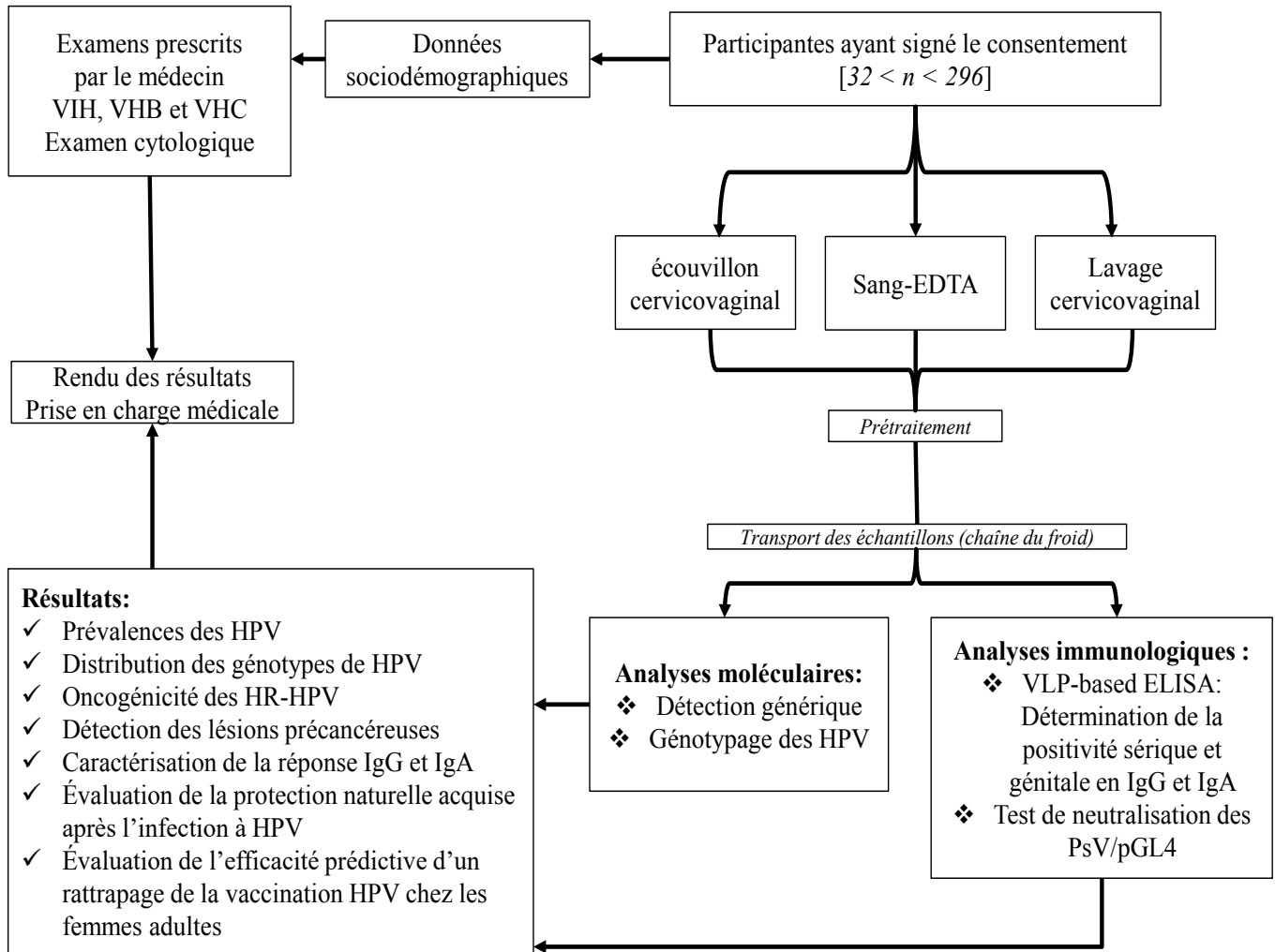


Figure 6. Diagramme directionnel de la réalisation du protocole de recherche IMMIPAP

c. Protocole HOMOPAP RCA

L'infection à Papillomavirus humain à haut risque (HR-HPV) demeure une grande préoccupation pour les hommes africains qui ont des relations sexuelles avec des hommes (HSH), en particulier ceux infectés par le VIH qui constituent le groupe le plus à risque pour l'infection à HR-HPV (Keshinro et al., 2016). Cependant, l'épidémiologie de l'infection à HPV chez les HSH en Afrique sub-Saharienne demeure très mal connue car peu documentée. Néanmoins, les observations faites dans quelques pays Africains au sein de la population HSH démontrent que cette population constitue un groupe de transmission de plusieurs infections sexuellement transmissibles et donc très à risque pour l'infection anale à HR-HPV. D'autre part, ces observations laissent supposer que la distribution des génotypes de HPV circulant au sein de cette population pourrait être relativement différente à celle observée dans la population générale, et dans d'autres continents (Müller et al., 2016; Nowak et al., 2016). Afin de vérifier ces hypothèses, nous avons dressé, dans le protocole de recherche

observationnelle et transversale HOMOPAP RCA, une estimation de la prévalence de l'infection anale à HR-HPV et identifié les facteurs de risque associés au sein d'un échantillon de HSH vivant à Bangui, en République Centrafricaine (RCA).

- **Critères d'inclusion :**
 - Hommes ayant des rapports sexuels avec des hommes (HSH), majeurs (≥ 18 ans)
 - Résider à Bangui en République Centrafricaine
 - Être approuvé par ses pairs comme étant véritablement un HSH
 - Avoir un suivi médical d'au moins trois mois
 - Avoir signé le formulaire du consentement éclairé.
- **Critères d'exclusion :**
 - Refus de participer à l'étude
 - Être hétérosexuel
 - Être mineur (< 18 ans).
- **Prélèvement et prétraitement :**

Chaque HSH inclus dans l'étude a subi un écouvillonnage de la marge anale qui a été congelé (-80°C), avant d'être transporté en respectant la chaîne du froid au laboratoire de virologie de l'hôpital Européen Georges Pompidou.

Protocole HOMOPAP

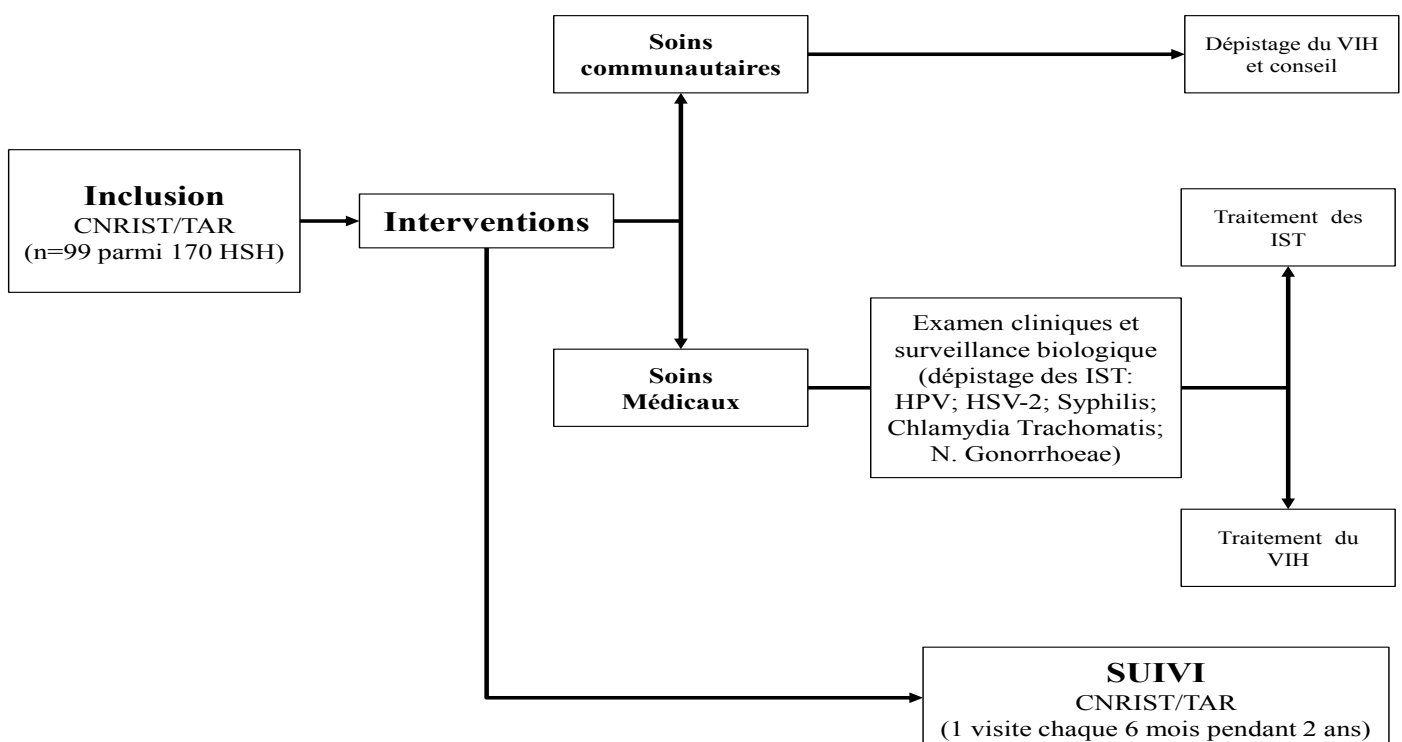


Figure 7. Diagramme directionnel de la réalisation du protocole de recherche HOMOPAP

3. Analyses de biologie moléculaire et sérologiques.

a. Analyses de biologie moléculaire

i. Extraction des acides nucléiques, détection et génotypage des HPV

Afin de réaliser l'amplification des ADN viraux pour le diagnostic moléculaire de l'infection à HPV dans les populations d'étude, les différents échantillons biologiques collectés ont été soumis à une extraction d'ADN à l'aide du kit DNeasy Blood and Tissue (Qiagen, CA, Etats-Unis) selon les instructions du fournisseur.

Le diagnostic moléculaire de l'infection à HPV a été effectué par le kit de PCR en temps réel Anyplex II HPV28 (Seegene, Séoul, Corée du sud). Le kit Anyplex permet la détection de 28 génotypes de HPV différents incluant 13 HR-HPV (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59 et -68), 9 LR-HPV (HPV-6, -11, -40, -42, -43, -44, -53, -54 et 70) et 6 génotypes considérés comme possiblement oncogéniques (HPV-26, -61, -66, -69, -73 et -82).

b. Analyses sérologiques

Les papillomavirus humains sont des virus quasiment impossibles à cultiver avec les technologies actuelles de façon à obtenir des rendements suffisamment grands pour permettre leur étude *in vitro*. Ainsi, l'étude des interactions immuno-virologiques entre le virus et son hôte ne peut être réalisée qu'à l'aide de pseudovirus (PsV) ou des « virus-like particles (VLP) » artificielles.

i. Production des antigènes viraux pour les analyses sérologiques : [HPV major capsid antigen L1 Virus-like particles (VLP)]

Pour la réalisation de cette étude, des VLP dérivant de 9 génotypes de HPV (HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-45, HPV-52, HPV-58 et HPV-68) ont été construites à l'aide de plasmides vecteurs biscistroniques (p16sheLL, p18sheLL, p31sheLL, pVITRO-HPV33L1L2, p35sheLL, p45sheLL, p52sheLL, p58sheLL and pVITRO-HPV68L1L2) incorporant chacun d'eux une copie des gènes codant pour la protéine majeure (L1) et la protéine mineure (L2) de la capsid virale de chacun des 9 génotypes de HPV. Les plasmides p16sheLL, p18sheLL, p45sheLL, p52sheLL and p58sheLL nous ont gracieusement été offert par monsieur John Schiller (Addgene plasmid # 37320; # 37321; # 37322; # 37323; # 46950; # 37324) (Buck et al., 2006; Roberts et al., 2007; Kondo et al., 2007; Kondo et al.,

2008). Le plasmide p35sheLL nous a été fourni par Simon Beddows (Addgene plasmid # 40626) (Draper et al., 2011) et les plasmides provenaient de monsieur Richard Roden (Addgene plasmid # 52493 and # 52587) (Kwak et al., 2014). Tous les plasmides étaient disponibles sur le centre de dépôt des séquences nucléiques Addgene (www.addgene.org).

La production des stocks de VLP a été réalisée telle que décrit précédemment (Buck & Thompson, 2007), à l'aide du protocole de production des vecteurs viraux HPV (<https://ccrod.cancer.gov/confluence/display/LCOTF/PseudovirusProduction>) édité par le Laboratoire d'Oncologie cellulaire du Centre pour la recherche sur le cancer (National cancer institute, National Institute of Health, Rockville Pike, Bethesda, Maryland, Etats-Unis). En résumé, les cellules 293TT ont été transfectées avec chacun des plasmides L1L2 et incubées dans du milieu DMEM (Dulbecco's Modified Eagle Medium) contenant 10% de SVF (sérum de veau fœtal), pendant 72 heures à 37°C. Les cellules ont ensuite été lysées dans un tampon de lyse (0.5% Nonidet™ P 40 Substitute/1X PBS, Sigma-Aldrich, Saint-Louis, Missouri, Etats-Unis) avant de subir 3 cycles (15 secondes/cycle) de sonication afin de rompre les membranes nucléaires, libérant ainsi les VLPs. Les extraits nucléaires obtenus ont ensuite été purifiés par ultracentrifugation à 30.500 tours/minute (SW 32 Ti Swinging-Bucket rotor, Beckman Coulter, Inc., Brea, CA, Etats-Unis) pendant 26 heures à 4°C dans un gradient (1,380, 1,338, 1,272, 1,164 g/mL) de chlorure de Césium (ClCs). La fraction contenant les VLPs est collectée et solubilisée dans 300µL de Dulbecco's Phosphate-Buffered Saline (DPBS) et conservée à 4°C en attendant les analyses sérologiques.

En outre, le contrôle qualité de la production des VLPs a été effectué par observation des pseudo-particules virales au microscope électronique à transmission (Jeol 400), à un grossissement x50000, après coloration négative à l'acétate d'uranyle à 1,5% (Service de Microscopie, Université François Rabelais de Tours, Tours, France). Les aliquotes de VLPs obtenus ont également été analysés par électrophorèse sur gel de polyacrylamide (SDS-PAGE) afin de s'assurer de la bonne production des protéines L1 de chaque génotype. L'anticorps monoclonal anti-HPV-16 [CamVir 1, ab69] (Abcam, Cambridge, Royaume-Unis) a été utilisé afin de s'assurer de la réactivité spécifique des VLP-16 et VLP-31 (génotype phylogénétiquement très proche du HPV-16) uniquement avec les anticorps anti-HPV-16 et anti-HPV-31. De même, l'anticorps monoclonal anti-HPV-18 L1 [abx110595] (Abxela Ltd, Cambridge, Royaume-Unis) a été utilisé comme contrôle qualité pour les VLP-18. Enfin, l'anticorps monoclonal anti-HSV1/HSV2 (*herpes simplex virus* type 1/2 Glycoprotein B), (ABIN457436, Antibodies-online, GmbH, Aachen, Germany) a été utilisé pour s'assurer de l'absence de réactivité croisée avec d'autre virus.

ii. Détermination de la séropositivité vis-à-vis de l'infection à HPV par Enzyme linked immunosorbent assay (ELISA)

Une analyse sérologique ELISA indirecte utilisant comme antigène les VLPs de chacun des 9 génotypes de HPV produit a été réalisée sur des échantillons de sérum et la partie acellulaire du lavage vaginal de chaque femme incluse. En bref, des plaques de microtitration ELISA à 96 puits (MaxiSorp™ NUNC F96, Thermo Fisher Scientific, Waltham, MA, Etats-Unis) ont été revêtues avec 100 µL de chaque VLP purifiée (VLP-16, VLP-18, VLP-31, VLP-33, VLP-33, VLP-33 -35, VLP-45, VLP-52, VLP-58 et VLP-68) dilués au 1:10 dans du DPBS (Thermo Fisher Scientific, Waltham, MA, Etats-Unis) et stockés pendant une nuit à 4 ° C. Chaque puits a été saturé avec 200 µL de PBS contenant 1% de sérum de veau fœtal (Dominique Dutscher SAS, Brumath, France), puis les plaques ont été incubées pendant 1 heure à température ambiante avant d'être vigoureusement décantées pour éliminer les antigènes en excès. Les sérums et les lavages génitaux appariés de chaque femme ont ensuite été dilués (1:10 et 1: 100 pour le lavage génital et le sérum, respectivement) dans du PBS et chargés dans les puits correspondants. Les plaques ont ensuite été incubées pendant 1 heure à l'obscurité, à 37 ° C. Après chaque étape d'incubation, les plaques ont été lavées 4 fois avec du PBS contenant 0,1% de Tween® 20 (Sigma-Aldrich, Saint-Louis, Missouri, Etats-Unis). Ensuite, les plaques ont été incubées à 37 ° C, dans l'obscurité, pendant 1 heure avec un anticorps monoclonal de souris anti-immunoglobulines (IgG) humaine conjugué à la peroxydase de raifort (HRP) [ab7499] (Abcam, Cambridge, Royaume-Unis) dilué dans du PBS (1:6000, selon les recommandations du fabricant) afin de détecter des IgG génitaux et sériques spécifiques à chaque VLP. Après les étapes d'incubation et de lavage, 100 µL de substrat enzymatique [peroxyde d'hydrogène (H₂O₂) Sigma-Aldrich, Saint-Louis, Missouri, États-Unis] et de chromogène (ortho-phénylènediamine, Sigma-Aldrich) ont été ajoutés dans des puits et les plaques ont été placées dans l'obscurité pendant une période d'incubation finale de 30 minutes pour permettre le développement de la chromogénèse. Les réactions de chromogénèse ont ensuite été arrêtées avec 0,1 M d'acide sulfurique (H₂SO₄, Thermo Fisher Scientific, Waltham, MA, Etat-Unis) et la densité optique (DO) associée a été lue entre 450 et 620 nm sur un lecteur de microplaques (ETIMAX 3000, DiaSorin, Saluggia, Italie). Tous les échantillons ont été testés en triple pour chaque type de VLP.

Un panel de sérums de 20 enfants français de moins de 5 ans a été utilisé comme contrôle négatif pour les tests sérologiques. En outre, un pool de sérums provenant d'individus ayant reçu les trois doses du vaccin Gardasil-9® (Merck & Co. Inc., New Jersey, États-Unis) a

constitué le contrôle positif de l'essai sérologique pour chacun des sept types de HPV à haut risque inclus dans le Gardasil-9® (VLP de HPV-6, -11, -16, -18, -31, -33, -45, -52 et -58).

II. Résultats

A. Épidémiologie moléculaire de l'infection à HPV au sein de populations africaines à risque

1. Forte prévalence de l'infection cervicale à Papillomavirus humains à haut-risque, en particulier par ceux couverts par le vaccin prophylactique Gardasil-9, au sein d'une population de femmes adultes vivant à N'Djamena, au Tchad.

Cette partie est basée sur l'article intitulé "**High prevalence of cervical high-risk human papillomavirus infection mostly covered by Gardasil-9 prophylactic vaccine in adult women living in N'Djamena, Chad**", paru le 3 juin 2019 dans le journal "*Public Library of Science (Plos One)*". Cette partie traite de l'épidémiologie de l'infection à Papillomavirus humains (HPV) et les principaux facteurs de risque associés, au sein d'une population de femmes adultes asymptomatiques vivant à N'Djamena, au Tchad.

Avant-propos.

Rationnel et objectif de l'étude. Avec plus de 75.000 nouveaux cas et près de 50.000 décès enregistrés chaque année, le cancer du col de l'utérus associé à l'infection persistante à HR-HPV représente le premier cancer enregistré dans la population féminine dans plusieurs pays d'Afrique sub-saharienne (Mboumba Bouassa et al., 2017).

Au Tchad, un pays de plus de 15 millions d'habitants, avec plus de 3 millions de femmes âgées de plus de 25 ans (INSEED 2016; INSEED 2016), aucun progrès significatif n'a été réalisé jusqu'à présent dans la prévention contre le cancer du col de l'utérus (Mortier et al., 2016). Seule une étude pilote évaluant la faisabilité du dépistage des lésions précancéreuses par la cytologie au sein de la population de femmes infectées par le virus de l'immunodéficience humaine (VIH) vivant au Tchad a été réalisée (Mortier et al., 2016). Cette étude montrait que les femmes infectées par le VIH constituaient une population très à risque pour la survenue des lésions cervicales de bas et haut-grade, suggérant indirectement un fort et insoupçonné taux d'infection cervicale par les HPV au sein de cette population en particulier, et même au sein de la population Tchadienne en général (Mortier et al., 2016). En outre, la prévention primaire par la vaccination par le vaccin prophylactique Gardasil-9 pourrait constituer une des meilleures options de prévention pour la population féminine vivant au Tchad dont une grande majorité n'a pas un libre accès au diagnostic secondaire et au traitement des lésions cervicales. Malheureusement, aucune donnée épidémiologique, sur la prévalence et la distribution des géotypes de HR-HPV circulant dans la population

générale au Tchad, n'est disponible à ce jour. Aussi, au courant de l'année 2018, nous avons conduit une étude transversale descriptive et quantitative, dans le but d'estimer la prévalence et les facteurs de risque associés à l'infection cervicale à HR-HPV au sein d'une population de femmes adultes asymptomatiques vivant à N'Djamena, la capitale du Tchad.

Méthodologie. Cinq (5) des dix principaux arrondissements de N'Djamena ont été sélectionnés de façon aléatoire. Des éducateurs autochtones, intervenant dans les églises communautaires et les réseaux associatifs des différents arrondissements de la capitale ont contacté et invité des femmes à participer à l'étude et à se rendre au site d'inclusion, la clinique pour la santé sexuelle des femmes "*La Renaissance Plus*", où leurs données sociodémographique et comportementales ont été collectées. La détection de l'infection à HPV et le génotypage des virus détectés ont été réalisés à partir d'écouvillons endocervicaux en utilisant le système de PCR en temps réel multiplex Anyplex II HPV28 genotyping test (Seegene, Seoul, Corée du Sud).

Résultats. Un total de 253 femmes (âge moyen : 35 ans, intervalle : 25-65 ans), parmi lesquelles 3,5% étaient infectées par le VIH, ont été prospectivement inclus dans l'étude durant l'année 2018. La prévalence de l'infection à HPV était de 22,9%, parmi lesquelles 68,9% étaient causées par des HR-HPV et 27,6% par plusieurs génotypes à la fois. Ce qui fait un total 15,8% [Intervalle de confiance à 95% (IC 95%) : 11,3-20,3] d'infections causées par des HR-HPV. Les génotypes à haut-risque les plus détectés étaient HPV-58, HPV-35, HPV-56, HPV-31, HPV-16, HPV-45, HPV-52 et HPV-18. Les HPV ciblés par le vaccin prophylactique Gardasil-9 représentaient près de 70% (67,5%) des infections enregistrées et HPV-58 était le plus fréquemment détecté. L'infection à VIH était le principal facteur risque, fortement associé à la présence de tout type de HPV [Odds ratio ajusté (ORa) : 17,4], aux infections cervicales par plusieurs génotypes à la fois (ORa : 8,9), aux infections par des HR-HPV (ORa : 13,2) et aux infections par plusieurs génotypes HR-HPV (ORa : 8,4).

Conclusions. Ces observations font ressortir la part importante et non suspectée de l'infection à HR-HPV dans la population féminine vivant au Tchad. Cette étude pointe également le risque potentiel de survenue de lésions cervicales précancéreuses et des cancers associés à l'infection à HPV dans une part importante de la population féminine Tchadienne. Enfin, le fort taux de circulation de génotypes ciblés par le vaccin Gardasil-9 constitue un rationnel pertinent pour l'implémentation de la vaccination par le Gardasil-9 chez les jeunes adolescentes Tchadiennes, comme outil efficace pour la prévention primaire contre l'infection à HPV et le cancer du col de l'utérus au Tchad.

RESEARCH ARTICLE

High prevalence of cervical high-risk human papillomavirus infection mostly covered by Gardasil-9 prophylactic vaccine in adult women living in N'Djamena, Chad

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Abstract

Background

We conducted in 2018 a descriptive, quantitative, population-based, cross-sectional survey estimating the prevalence of cervical high-risk human papillomavirus (HR-HPV) infection and associated risk factors among adult women living in N'Djamena, Chad.

Methods

Five of the 10 districts of N'Djamena were randomly selected for inclusion. Peer educators contacted adult women in community-churches or women association networks to participate in the survey and come to the clinic for women's sexual health "La Renaissance Plus", N'Djamena. Medical, socio-demographical and behavioral informations were collected. HPV DNA was detected and genotyped in endocervical swab using Anyplex II HPV28 genotyping test (Seegene, Seoul, South Korea).

Results

253 women (mean age, 35.0 years; range, 25–65) including 3.5% of HIV-positive women were prospectively enrolled. The prevalence of HPV infection was 22.9%, including 68.9% of HR-HPV infection and 27.6% being infected with multiple genotypes, providing a total HR-HPV prevalence of 15.8% (95% CI%: 11.3–20.3). The most prevalent HR-HPV

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genotypes were HPV-58, HPV-35, HPV-56, HPV-31, HPV-16, HPV-45, HPV-52 and HPV-18. HPV types targeted by the prophylactic Gardasil-9 vaccine were detected in nearly 70% (67.5%) and HPV-58 was the most frequently detected. HIV infection was a risk factor strongly associated with cervical infection with any HPV [adjusted Odds ratio (aOR): 17.4], multiple types of HPV (aOR: 8.9), HR-HPV (aOR: 13.2) and cervical infection with multiple HR-HPV (aOR: 8.4).

Conclusion

These observations highlight the unsuspected high burden of cervical HR-HPV infection in Chadian women, and point the potential risk of further development of HPV-associated cervical precancerous and neoplastic lesions in a large proportion of women in Chad. The high rate of preventable Gardasil-9 vaccine genotypes constitutes the rationale for introducing primary vaccine prevention against cervical cancer in young female adolescents living in Chad.

Introduction

Human papillomavirus (HPV) infection is the most common viral sexually transmitted infection (STI) worldwide and high-risk (HR)-HPV genotypes are responsible for 5.2% of all cancers worldwide, 2.2% of cancers in developed countries and 7.7% of all cancers in developing countries [1–3]. In sub-Saharan Africa, cervical cancer associated with persistent cervical HR-HPV infection is the most common cancer in women in many countries, with more than 75,000 new cases and nearly 50,000 deaths registered each year [4, 5]. According to the World Health Organization (WHO), cervical cancer will kill annually more than 443,000 women around the world by 2030 and 98% of these deaths will occur in developing countries and especially in Sub-Saharan Africa where HIV epidemic and other risk-factors are aggravating the burden of this cancer [5, 6]. Thus, cervical cancer has become progressively one of the main public health challenges to overcome in sub-Saharan Africa since the recent decades [7].

Although all HR-HPV genotypes have oncogenic potency, some of them are more frequently involved in cancers than others. These include HPV-16 and HPV-18, accounting for about 70% of all cervical cancers worldwide, and HPV-31, HPV-33, HPV-45, HPV-52, HPV-58 and HPV-68 which are among the ten most frequently isolated HR-HPV genotypes from cervical tumor biopsies [8, 9, 10]. The current 9-valent Gardasil-9 vaccine (Merck & Co. Inc., New Jersey, USA), targeting the seven primarily isolated HR-HPV genotypes from cervical cancers (HPV-16, -18, -31, -33, -45, -52 and -58) and the two primarily isolated low-risk (LR)-HPV (HPV-6 and HPV-11), has been developed according to these epidemiological data [10]. Thus, primary prevention of cervical cancer by vaccinating young girls at 10 to 14 years of age with Gardasil-9 vaccine prior sexual onset would protect them theoretically against most of the cervical HR-HPV infections [11, 12]. This primary prevention, combined with other prevention strategies (secondary and tertiary prevention) already in place in sub-Saharan Africa would overcome the high burden of cervical cancer in this continent [7, 13].

Before implementing vaccination with Gardasil-9 vaccine in African countries, it is essential to establish the epidemiological distribution of the main circulating genotypes in the general population. Indeed, these indications are critical because they allow evaluating the preventive efficiency of vaccination against HPV with the current HPV vaccine in specific

populations. A wide heterogeneity in the distribution of circulating HR-HPV genotypes exists from one country to another and also between two regions within the same country throughout the African continent [14]. Generally, the epidemiological landscape of HPV infection in Sub-Saharan Africa is mostly dominated by HR-HPV genotypes targeted by the Gardasil-9 vaccine [14–20]. Some countries in sub-Saharan Africa where a good match between prevalent HPV types and Gardasil-9 has been demonstrated are now implementing vaccination of adolescents, with support from international donors [7, 21].

In Chad, a country of around 15 million people, including more than 3 million women aged more than 25 year-old [22, 23], no significant progress has been realized until now in cervical cancer prevention [24, 25]. Only one pilot study assessing the feasibility of the cytology-based screening for cervical lesions in HIV-infected women has been conducted in Chad [25]. Thus, HIV-infected Chadian women were at high-risk for low and high-grade cervical lesions, suggesting indirectly a high and unsuspected burden of cervical HPV infection in Chad [25]. In addition, primary prevention by prophylactic vaccine could likely constitute one of the best options for many women in Chad, who will likely not have the luxury of diagnostic exams and care. Unfortunately, epidemiological data on the distribution of circulating HR-HPV genotypes in the general population in Chad are lacking [26, 27]. Herein, we designed and carried out a cross-sectional study to assess the prevalence and genotypes distribution of cervical HPV infection and associated risk factors in adult women living in N'Djamena, the capital city of Chad.

Material and methods

Study design

The study was a descriptive, quantitative, population-based, cross-sectional survey, using a face-to-face questionnaire to collect data in 2018 among adult women living in N'Djamena, Chad.

The laboratory protocol of this study was been deposited in protocols.io website and is available at: [dx.doi.org/10.17504/protocols.io/wgefbbte](https://doi.org/10.17504/protocols.io/wgefbbte).

Enrolment and selection criteria

The capital city N'Djamena comprises 10 districts, which include a variable number of neighborhoods. Twenty-three sites in neighborhoods of 5 out of the 10 districts randomly selected were further chosen for study inclusion, as depicted in the Fig 1. In each inclusion site, peer educators contacted adult women in community-churches or women association networks during a one-month period and proposed that they participate in the study after an oral explanation and collective awareness sessions on the objectives of the survey, mainly focused on sensitization on cervical cancer and prevention strategies against cervical HPV acquisition. After oral consent, the selected women were invited, with paid transportation, to come to the clinic “*La Renaissance Plus*”, N'Djamena, which is one of the main settings for women's sexual health in Chad, and to participate in the study. Adult women living in N'Djamena regularly attend the clinic “*La Renaissance Plus*” for gynecological examinations and for obstetrical services.

The inclusion criteria were agreeing to participate in the study, having given signed informed consent, being aged between 25–65 years, being sexually active, having no genital troubles at physical examination and having completed the questionnaire. Exclusion criteria included age less than 25 year-old and more than 65 year-old and not willing to participate to the study or to answer the face-to-face questionnaire to collect data.

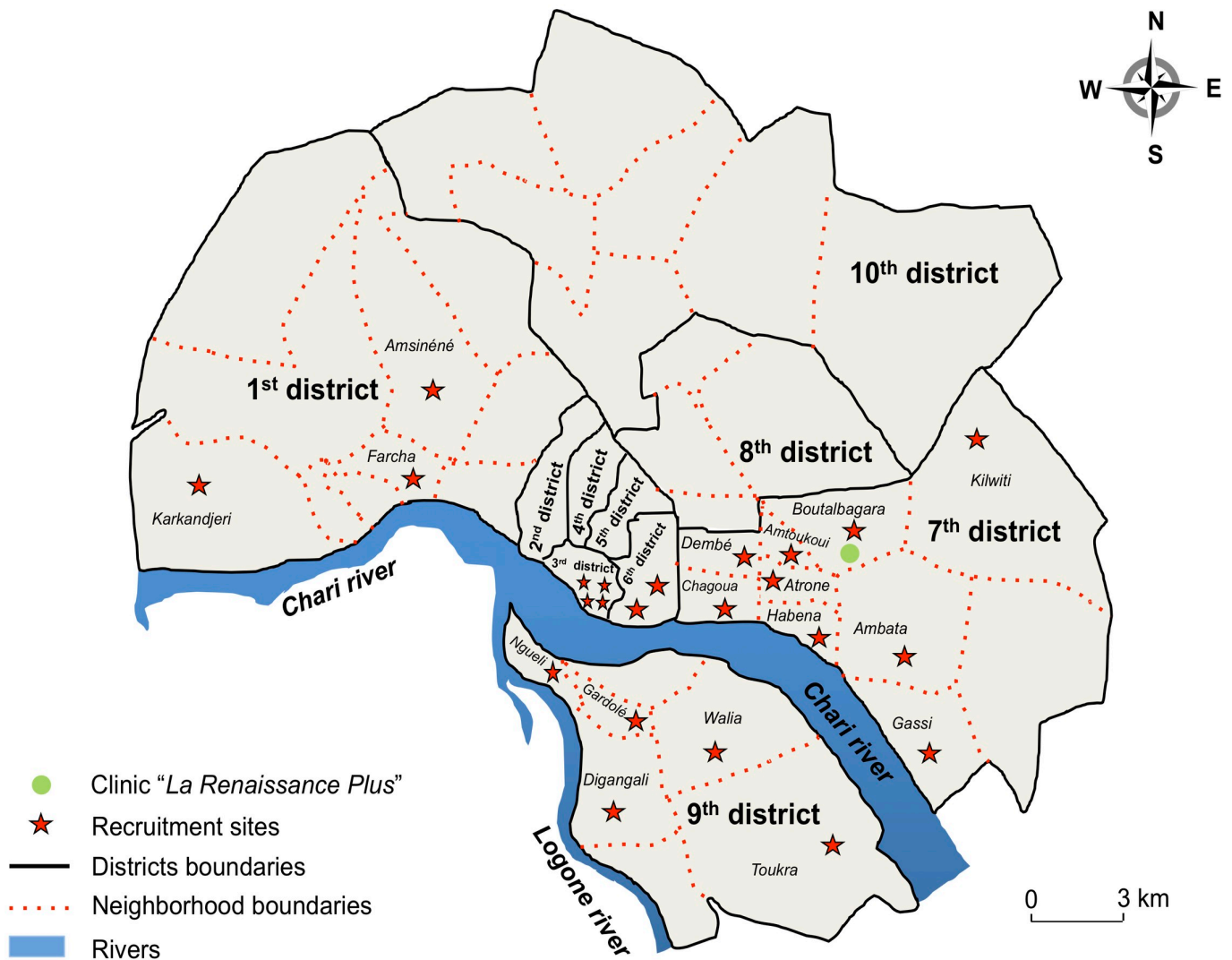


Fig 1. Map showing the location of 23 inclusion study sites in N'Djamena, Chad, including 5 out of 10 districts randomly selected in which neighborhoods were further chosen for awareness of the study [1st district: Farcha, Amsiné, Karkandjeri; 3rd district: Gardolé, Ardep Djoumal, Kabalaye, Sabangali; 6th district: Moursal, Paris-Congo; 7th district: Ambata, Amtoukoui, Atrone, Boutalbagara, Chagoua, Dembé, Gassi, Habena, Kilwiti; 9th district: Digagali, Gardolé, Ngueli, Tourka, Wali]. In each inclusion site, peer educators contacted adult women in community-churches or women associations during a one-month period and proposed them to be included in the study after an oral explanation and collective awareness sessions on the objectives of the survey, mainly focused on information on cervical cancer.

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After signed the informed consent form, the selected women benefited from free HIV and hepatitis B (HBV) and C (HCV) testing, by multiplex HIV/HCV/HBsAg immunochromatographic rapid test (Biosynex, Strasbourg, France) [28], clinical services including gynecological examination, family planning counseling, STIs diagnosis, laboratory analysis where necessary and appropriate treatment for those suffering from gynecologic disorders or from genital or HIV infections. All women received an information session on HIV and STIs, and completed a face-to-face questionnaire.

At inclusion, a standardized interview was conducted at the clinic "La Renaissance Plus", by experienced counsellors using a face-to-face questionnaire to collect socio-demographic characteristics and behavioral data, including age, marital status, social occupation, education level, residence location in N'Djamena, history of STI, HIV status and also sexual behavioral characteristics such as the number of lifetime sexual partners and the age at first sexual intercourse.

Samples and processing

After completing the socio-demographic data collection questionnaire, a nurse performed cervicovaginal sampling using a flocked swab (Copan Diagnostic Inc., California, USA). Briefly, specimens for molecular testing were obtained by inserting the swab into the vaginal canal until the cervix mucosa, gently rotating 5 times and then removed and immediately placed into its container and frozen at -80°C before DNA extraction procedure. Finally, cervicovaginal swab were transported in frozen ice packs, to the virology laboratory of the *hôpital Européen Georges Pompidou*, Paris, France, for molecular analyses.

HPV detection and genotyping

DNA was extracted from the cervical swab specimen using the DNeasyBlood and Tissue kit (Qiagen, Hilden, Germany) as recommended by the manufacturer. After extraction, DNA was concentrated and eluted in 100 to 200 μL of the kit elution buffer before genotyping.

The detection and the genotyping of HPV were carried out using the real-time PCR assay Anyplex II HPV28 (Seegene, Seoul, South Korea), [29]. According to the HPV classification nomenclature provided by the International Agency for Research on Cancer (IARC) [30], Anyplex II HPV28 allows to distinguish 28 HPV genotypes, including 13 high-risk types (HR-HPV -16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68), 9 low-risk (LR) types (LR-HPV -6, -11, -40, -42, -43, -44, -53, -54 and -70) and then, 6 genotypes classified as probably carcinogenic (HPV-26, -61, -66, -69, -73 and -82). Briefly, 5 μL of swab-extracted DNA are added into two reaction mixtures (20 μL) containing each other, one of the primers sets A and B [29]. The DNA amplification and the genotyping process are carried out in 2 reactions performed on the CFX96 real-time PCR instrument (Bio-Rad, Marnes-la-Coquette, France) [29]. The Anyplex II HPV28 genotyping test has been found to be suitable for HPV detection and genotyping in cervical secretions [29, 31–34]. Data recording and interpretation were automated with Seegene viewer software (Seegene), according to the manufacturer's instructions. A swab sample was considered positive for any HPV if containing any of the 28 types targeted by the Anyplex II HPV28 detection test; positive for multiple HPV when containing at least 2 types of the 28 HPV types included in genotypic test; HR-HPV positive and multiple HR-HPV positive when containing respectively at least 1 HR-HPV type and at least 2 high-risk types belonging to the 19 high-risk types targeted by the Anyplex II HPV28 molecular test, irrespective of the presence of LR-HPV. The virology laboratory was accredited in 2013 by the *Comité Français d'Accréditation* (COFRAC) according to the ISO 15189 Norma for the biological markers "HPV detection" and "HPV genotyping".

Statistical analyses

Data was entered into an Excel database and analyzed using IBM SPSS Statistics 20 software (IBM, SPSS Inc, Armonk, New York, USA). Means and standard deviations (SD) were calculated for quantitative variables and proportions for categorical variables. The results were presented as a 95% confidence interval (CI) using the Wilson score bounds. P-values (P) were calculated using Pearson's χ^2 test or Fisher's exact test for categorical variables and the non-parametric Mann-Whitney U -test for quantitative variables. Logistic regression models using univariate and multivariate analyses were performed to determine the association of each independent variable [*i.e.*, "age at inclusion", "HIV infection", "marital status", "social occupation", "education level", "number of lifetime sexual partners" and "age at first sexual intercourse"] with the HPV type-specific cervical infections (*i.e.*, genital infection by any type of HPV, multiple types of HPV, HR-HPV and multiple HR-HPV). All variables statistically significant ($P < 0.05$) in univariate analysis were computed into a multivariate logistic regression

analysis. Crude Odds ratio (cOR) and adjusted Odds ratio (aOR) were calculated, as appropriate along with their 95% CI. Finally, a risk factor was defined as an independent variable giving in univariate analysis a cOR (along with its 95% CI) strictly higher than “1” with a *P*-value lower than 0.05. An aOR (along with its 95% CI) strictly higher than “1” with a *P*-value lower than 0.05 defines a risk factor in multivariate analysis. A protective factor was defined as an independent variable for which the cOR (along with its 95% CI) is strictly lower than “1” with a *P*-value lower than 0.05 in univariate analysis. An aOR (along with its 95% CI) strictly lower than “1” with a *P*-value lower than 0.05 characterizes a protective factor in multivariate analysis [35].

Ethics statement

The study was formally approved by the Scientific Committee of the Faculty of Health Sciences of the University of N'Djamena, constituting the National Ethical Committee. All included women gave their informed signed consent to participate to the study. For each included woman, the record of the consent to participate to the study was documented on each questionnaire. This consent procedure was formally approved by the Ethical Committee.

All individual results of HPV detection and genotyping were given to each study participant, and women harboring cervical HR-HPV were further referred for diagnosis of HPV-related lesions and care at the clinic “*La Renaissance Plus*”. Furthermore, the study results have been *in extenso* reported to health authorities of Chad during the national congress of gynecologists and midwives, held from the 13th to 17th of November 2018 in the *Centre d'Etudes et de Formation pour le Développement* (CEFOD), N'Djamena, Chad.

Results

Characteristics of study population

From June to August 2018, 260 women from the 23 inclusion sites participated to the study [1st district (32, 12.4%): Farcha (n = 14), Amsinéné (n = 8), Karkandjeri (n = 10); 3rd district (54, 20.2%): Gardolé (n = 12), Ardep Djoumal (n = 19), Kabalaye (n = 10), Sabangali (n = 13); 6th district (47, 18.3%): Moursal (n = 23), Paris-Congo (n = 24); 7th district (61, 23.6%): Ambata (n = 5), Amtoukoui (n = 6), Atrone (n = 9), Boutalbagara (n = 5), Chagoua (n = 7), Dembé (n = 8), Gassi (n = 9), Habena (n = 9), Kilwiti (n = 3); 9th district (66, 25.5%): Digangali (n = 10), Gardolé (n = 7), Ngueli (n = 11), Tourka (n = 12), Walia (n = 26)].

After physical examination, 7 women were excluded because of genital troubles (vaginal discharge: 2; suspicion of STI: 2; genital bleeding: 3). Finally, a total of 253 women (mean age, 35.0 years; range, 25–65) referred to the clinic “*La Renaissance Plus*” were consecutively and prospectively included in the study and their socio-demographic, sexual behavior, clinical and biological characteristics are summarized in the [Table 1](#).

Using multiplex HIV/HCV/HBsAg rapid test, 9 study women (3.5%; 95% CI: 1.3–5.8) were diagnosed infected by HIV-1, 19 (7.5%; 95% CI: 4.3–10.8) by HBV (positivity for HBsAg) and 8 (3.2%; 95% CI: 1.1–5.3) were seropositive for HCV.

Several women (31.6%; 95% CI: 25.9–37.4) were young, aged from 25 to 29 years, engaged in life couple with a male partner (78.3%; 95% CI: 73.2–83.3), with a relatively high education level (32.1%; 95% CI: 26.3–37.7 and 30.4%; 95% CI: 24.7–36.1, in high school level and university, respectively); but most of them were unemployed (54.2%; 95% CI: 48.1–60.3). The majority of study women (83.8%; 95% CI: 79.3–88.4) reported having only one regular sexual partner in their life, while about 10% reported to have up to 5 different sexual partners. Generally, women included in this study became sexually active at 16 to 20 years of age (56.2%; 95% CI: 50.1–62.3), whereas some of them (30.1%; 95% CI: 24.4–35.7) started their sexual life

Table 1. Baseline characteristics of 253 adult women living in N'Djamena, in Chad.

Characteristics	Study women (N = 253)
	n (%) [95% CI]*
Age	
All age [mean (SD), years]	35.0 (9.9) (25–65 years)
25–29	80 (31.6) [25.9–37.4]
30–39	66 (26.1) [20.7–31.5]
40–49	69 (27.3) [21.7–32.7]
≥ 50	38 (15.1) [10.6–19.4]
HIV infection	9 (3.5) [1.3–5.8]
HBV infection	19 (7.5%) [4.3–10.8]
HCV infection	8 (3.2%) [1.1–5.3]
Past history of STI	10 (3.9) [1.5–6.3]
Marital status	
Single	15 (5.9) [3.1–8.8]
Living in couple	198 (78.3) [73.2–83.3]
Divorced	28 (11.1) [7.2–14.9]
Widow	10 (3.9) [1.5–6.3]
Occupation	
Student	36 (14.2) [9.9–18.5]
Unemployed	137 (54.2) [48.1–60.3]
Employed	78 (30.8) [25.2–36.5]
Education level	
Never schooled	50 (19.7) [14.8–24.6]
Elementary school	45 (17.8) [13.1–22.5]
High school	81 (32.1) [26.3–37.7]
University	77 (30.4) [24.7–36.1]
Sexual behaviors	
Age at sexual onset [years]	
< 16	76 (30.1) [24.4–35.7]
16–20	142 (56.2) [50.1–62.3]
> 20	35 (13.8) [9.6–18.1]
Number of sexual partners in the life	
One regular partner	208 (82.2) [77.5–86.9]
Several partners [1 to 5]	45 (17.8) [13.1–22.5]
HPV DNA detection and types	
HPV DNA in swab	58 (22.9) [17.7–28.1]
Multiple types of any HPV	16 (27.6) [16.1–39.1]
LR-HPV	25 (43.1) [30.4–55.8]
Probably oncogenic HPV	9 (15.5) [6.2–24.8]
HR-HPV	40 (68.9) [57.1–80.8]
Multiple types of HR-HPV among HR-HPV-positive swabs	10 (25.0) [11.6–38.4]
HPV-16	5 (8.6) [1.4–15.8]
HPV-18	4 (6.9) [0.4–13.4]
Any 4-valent vaccine types** among HPV-positive swabs	12 (20.7) [10.3–31.1]
Multiple 4-valent vaccine types among HPV-positive swabs	1 (1.7) [0.0–5.1]
Any 9-valent vaccine types*** among HPV-positive swabs	29 (50.0) [37.1–62.9]
Multiple 9-valent vaccine types among HPV-positive swabs	6 (10.3) [2.5–18.2]
9-valent vaccine HR-HPV types among HR-HPV-positive swabs	27 (67.5) [52.9–82.1]

(Continued)

Table 1. (Continued)

	Study women (N = 253)
9-valent vaccine HR-HPV types only among HR-HPV-positive swabs	20 (50.0) [34.5–65.5]
Non-vaccine HR-HPV types only among HR-HPV-positive swabs	13 (32.5) [17.9–47.1]
Both non-vaccine and 9-valent vaccine HR-HPV types	6 (10.3) [2.5–18.2]

*The frequency of each variable is presented with their 95% confidence interval in brackets;

**The 4-valent Gardasil-4 vaccine (Merck & Co. Inc., New Jersey, USA) is effective against HPV genotypes 6, 11, 16 and 18;

***The 9-valent Gardasil-9 vaccine (Merck & Co. Inc.) is effective against HPV genotypes 6, 11, 16, 18, 31, 33, 45, 52 and 58.

Nota bene: HPV DNA percentage was calculated among all the 253 study women; multiple types of HR-HPV positivity was calculated among swabs positive for HR-HPV DNA; the remaining HPV outcomes were calculated among swabs positive for HPV DNA.

95% CI: 95% confidence interval; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; STI: Sexual transmitted infection; HPV: Human papillomavirus; LR-HPV: Low-risk human papillomavirus; HR-HPV: High-risk human papillomavirus.

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earlier, before 16 years. Finally, none of the women included in this study had ever been screened for cervical cancer and nor vaccinated against HPV infection.

Prevalence of HPV detection and genotype distribution

Overall, 58 adult women were positive for genital HPV DNA giving a total HPV prevalence of 22.9% (95% CI: 17.7–28.1). Of those who were HPV DNA positive, 68.9% (40/58; 95% CI: 57.1–80.8) harbored cervical HR-HPV infection, providing an overall HR-HPV prevalence of 15.8% (95% CI: 11.3–20.3), as shown in the [Table 1](#). Genital infections with multiple HPV genotypes were frequent in study women (27.6%; 95% CI: 16.1–39.1). Furthermore, 25.0% (95% CI: 11.6–38.4) of HR-HPV positive specimens contained multiple HR-HPV genotypes with an average of 2.3 HR-HPV (range, 1 to 5) per cervical swab sample. The whole distribution of HPV genotypes in HPV-DNA positive cervical samples is detailed in the [Fig 2](#).

The Gardasil-9 vaccine HR-HPV type 58 was the predominant genotype (7/58; 12.1%), followed by the HR-HPV types 31, 35 and 56 and the LR-HPV types 42 and 44 with a prevalence of 10.3% (6/58). The 9-valent vaccine HR-HPV types 16, 45 and 52 and also the LR-HPV types 53 and 70 were present with a prevalence of 8.6% (5/58). These HPV genotypes were followed by the HR-HPV types 18, 51, 59 and 68, the LR-HPV types 6 and 54 and the probably oncogenic HPV types 73 and 82 with a prevalence of 6.9% (4/58). The HR-HPV-39 was present only in 3 women (5.2%) and a minority of study women were infected with LR-HPV types 40 and 43 (3.4%; 2/58) and HR-HPV-33 (1.7%; 1/58). Finally, none of the HPV positive samples was simultaneously positive for HPV-16 and HPV-18 ([Fig 2](#)).

Cervical HPV DNA according to socio-demographic and behavioral characteristics and HIV serostatus

Concerning the distribution of cervical HPV type-specific prevalence according to age groups, there was no significant difference between each of the 4 age groups. Even so, women aged from 25 to 29 years were most infected with any HPV types and LR-HPV types than both the 3 others age groups ([Fig 3A](#)). Likewise, women aged from 25 to 29 years and even those aged from 40 to 49 years harbored higher HR-HPV prevalence than women belonging to the age

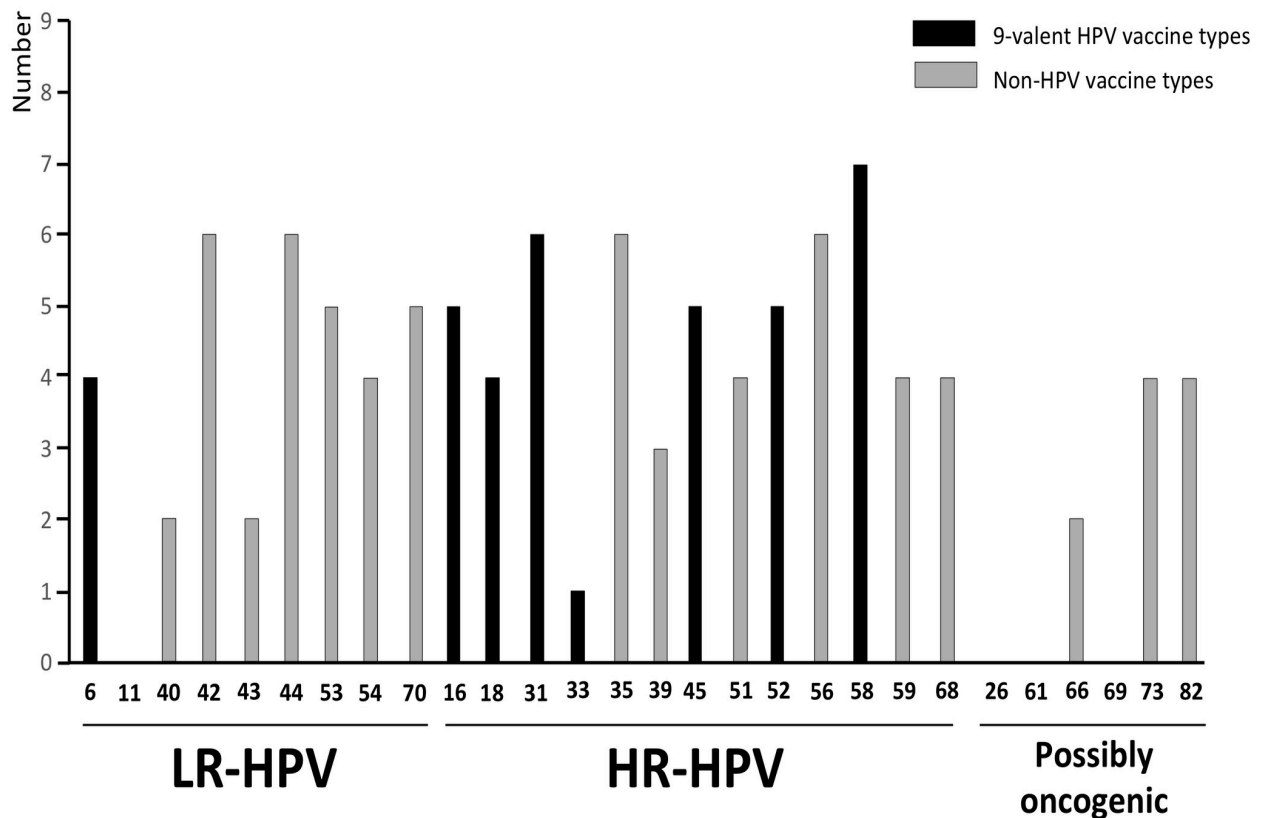


Fig 2. Distribution of HPV genotypes according to their inclusion in the 9-valent Gardasil-9 vaccine. Number of low-risk (LR) and high-risk (HR) HPV genotypes in 57 cervical samples positive for HPV DNA by molecular biology according to their possible prevention by 9-valent HPV vaccine among adult women (n = 253) living in N’Djamena, Chad. Nota bene. The 9-valent Gardasil-9 vaccine (Merck & Co. Inc., New Jersey, USA) is effective against HPV genotypes 6, 11, 16, 18, 31, 33, 45, 52 and 58.

<https://doi.org/10.1371/journal.pone.0217486.g002>

groups 30 to 39 years and those being 50 years of age and older. HPV types considered as probably carcinogenic were only present in women of 30 years of age and over (Fig 3A).

The distribution of cervical HPV type-specific prevalence according to the age of sexual intercourse onset is depicted in the Fig 3B. Women who had their first sexual intercourse between 16 to 20 years of age carried higher cervical HPV infection with any genotype (54.3%) compared to women who began their sexual onset below 16 years of age (29.8%) and women who started sexual intercourse after 20 years (15.8%), ($P = 0.00006$). High-risk types was similarly distributed with higher rates (36.2%) in women who started sexual intercourse between 16 to 20 years of age compared to women who had their sexual debut below 16 years (20.7%) and those who began after 20 years of age (12.1%), ($P = 0.007$).

The impact of HIV in HPV type-specific infection in the study was assessed. Seven out of the 9 HIV-infected women (77.8%) were positive for cervical HPV DNA and 85.7% (6/7) of these infections were caused by HR-HPV genotypes, with the predominant genotype being HPV-56 (42.8%; 3/7). Moreover, when compared to HIV-negative, HIV-positive women were significantly more infected with any type of HPV (77.8%; 95% CI: 50.6–100.0 versus 20.5%; 95% CI: 15.4–25.6; $P = 0.0005$), HR-HPV types (66.7%; 95% CI: 35.8–97.5 versus 13.5%; 95% CI: 9.2–17.8; $P = 0.0005$), multiple infections with any type of HPV (33.3%; 95% CI: 2.5–64.1 versus 4.9%; 95% CI: 2.2–7.6; $P = 0.01$) and multiple HR-HPV types (22.2%; 95% CI: 0.0–49.4 versus 3.7%; 95% CI: 1.3–6.1; $P = 0.03$) (Table 2).

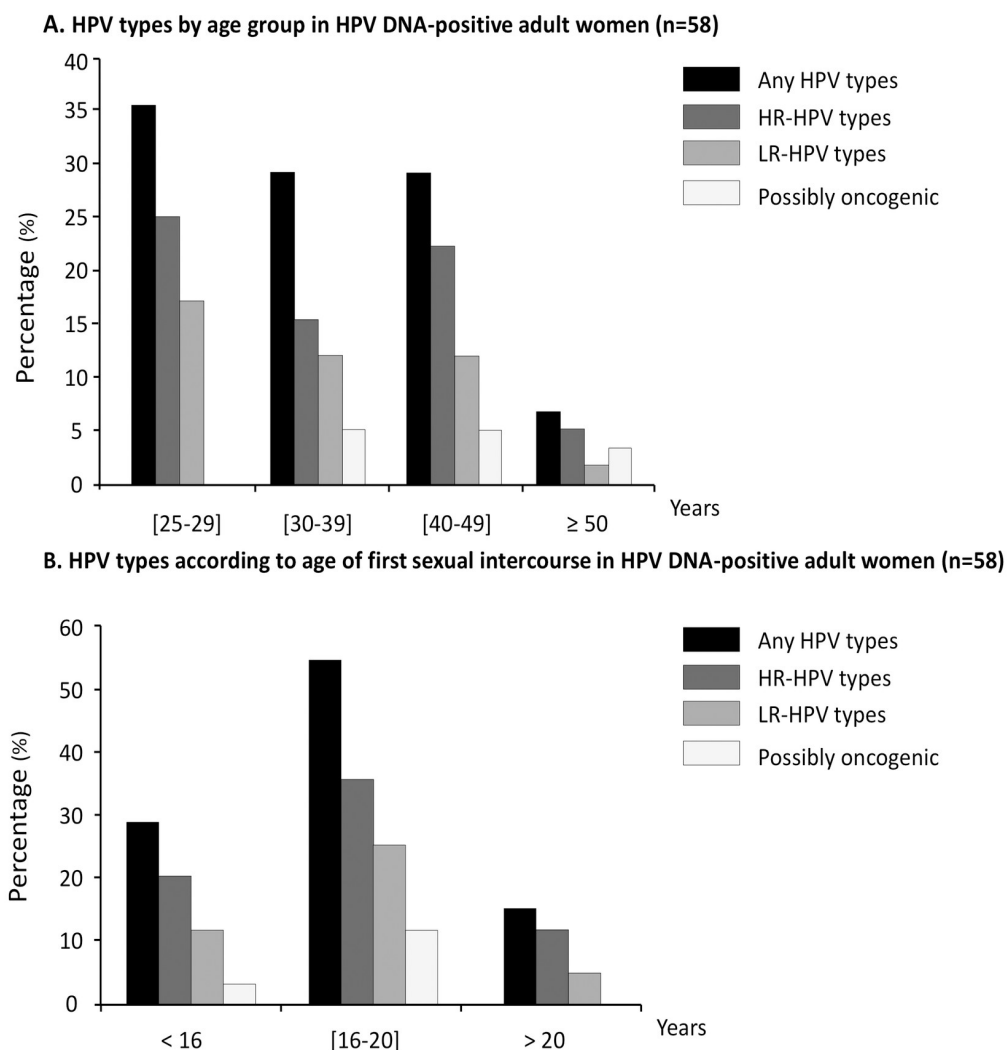


Fig 3. Distribution of HPV types in 57 adult women. A. According to age groups; **B.** According to age at first sexual intercourse.

<https://doi.org/10.1371/journal.pone.0217486.g003>

Predictive risk factors for cervical HPV shedding by logistic regression analyses

The associations between each one of the type-specific HPV infections with their potential predictive risk factors were assessed by logistic regression analysis, as shown in the [Table 2](#).

In univariate analysis, the variable “HIV infection” was significantly associated with cervical infection with any type of HPV (cOR: 13.2, 95% CI: 2.7–65.7%; $P = 0.001$), multiple types of HPV (cOR: 8.9, 95% CI: 2.0–39.6; $P = 0.014$), HR-HPV (cOR: 12.4, 95% CI: 2.9–51.7%; $P = 0.001$) and cervical infection with multiple HR-HPV (cOR: 8.4, 95% CI: 1.5–47.2%; $P = 0.044$). Likewise, the variable “being student” was statistically associated with the cervical carriage of any type of HPV (cOR: 2.2, 95% CI: 1.1–4.6%; $P = 0.042$) and HR-HPV (cOR: 2.4, 95% CI: 1.1–5.5%; $P = 0.034$). Another statistical association seems to emerge between the variables “being 50 years of age or older” and getting infected with any HPV (cOR: 0.4, 95% CI: 0.08–1.08%; $P = 0.057$). “Being 50 years of age or older” seems to have a slightly protective effect against cervical infection with any HPV, but the statistical power of this protective effect was canceled.

Table 2. Logistic regression analyses for cervical HPV-associated risk factors in 253 adult women living in N'Djamena, Chad.

Risk factors	Any HPV (N = 58)						Multiple HPV (N = 16)						HR-HPV (N = 40)						Multiple HR-HPV (N = 10)					
	n (%)	cOR (95%CI)	P*	aOR (95%CI)	P	n (%)	cOR (95%CI)	P*	aOR (95%CI)	P	n (%)	cOR (95%CI)	P*	aOR (95%CI)	P	n (%)	cOR (95%CI)	P*	aOR (95%CI)	P				
Age (years)																								
25–29	20 (34.5)	Reference	-	NA**	NA	6 (37.5)	Reference	-	NA	NA	15 (37.5)	Reference	-	NA	NA	3 (30.0)	Reference	-	NA	NA				
30–39	17 (29.3)	1.2 (0.6–2.4)	0.071	NA	NA	3 (18.8)	0.6 (0.2–2.3)	0.769	NA	NA	9 (22.5)	0.8 (0.4–1.8)	0.573	NA	NA	3 (30.0)	1.2 (0.3–4.9)	0.724	NA	NA				
40–49	17 (29.3)	1.1 (0.6–2.2)	0.087	NA	NA	5 (31.2)	1.2 (0.4–3.7)	0.773	NA	NA	13 (32.5)	1.4 (0.7–2.8)	0.418	NA	NA	3 (30.0)	1.1 (0.3–4.6)	1.00	NA	NA				
≥ 50	4 (6.9)	0.4 (0.08–1.08)	0.057	NA	NA	2 (12.5)	0.8 (0.2–3.7)	0.771	NA	NA	3 (7.5)	0.4 (0.1–1.4)	0.147	NA	NA	1 (10.0)	0.6 (0.1–5.0)	1.00	NA	NA				
HIV infection																								
No	51 (87.9)	Reference	-	Reference	-	13 (81.2)	Reference	-	Reference	-	34 (85.0)	Reference	-	Reference	-	8 (80.0)	Reference	-	Reference	-				
Yes	7 (12.1)	13.2 (2.7–65.7)	0.001	17.4 (3.2–94.9)	0.001	3 (18.8)	8.9 (2.0–39.6)	0.014	8.9 (2.0–39.6)	0.004	6 (15.0)	12.4 (2.9–51.7)	0.001	13.2 (3.1–56.6)	< 0.001	2 (20.0)	8.4 (1.5–47.2)	0.044	8.4 (1.5–47.2)	0.015				
Marital status																								
Single	4 (6.9)	1.2 (0.4–4.0)	0.753	NA	NA	1 (6.2)	1.1 (0.1–8.6)	0.955	NA	NA	2 (5.0)	0.8 (0.2–3.7)	1.00	NA	NA	1 (10.0)	1.8 (0.2–15.4)	0.464	NA	NA				
Living in couple	44 (75.9)	Reference	-	NA	NA	14 (87.5)	Reference	-	NA	NA	33 (82.5)	Reference	-	NA	NA	8 (80.0)	Reference	-	NA	NA				
Divorced	2 (3.4)	0.8 (0.08–4.5)	0.822	NA	NA	0 (0.0)	NA	1.00	NA	NA	1 (2.5)	0.6 (0.1–4.7)	1.00	NA	NA	0 (0.0)	NA	1.00	NA	NA				
Widowed	8 (13.8)	1.4 (0.6–3.4)	0.451	NA	NA	1 (6.2)	0.5 (0.1–4.1)	1.00	NA	NA	4 (10.0)	0.9 (0.3–2.7)	1.00	NA	NA	1 (10.0)	0.9 (0.1–7.3)	1.00	NA	NA				
Occupation																								
Unemployed	29 (50.0)	Reference	-	Reference	-	9 (56.2)	Reference	-	NA	NA	21 (52.5)	Reference	-	1.5 (0.1–28.9)	0.805	6 (60.0)	Reference	-	NA	NA				
Student	13 (22.4)	2.2 (1.1–4.6)	0.042	3.2 (0.2–42.9)	0.381	5 (31.2)	3.0 (0.98–9.3)	0.059	NA	NA	10 (25.0)	2.4 (1.1–5.5)	0.034	3.5 (0.2–66.6)	0.411	3 (30.0)	2.7 (0.7–11.1)	0.145	NA	NA				
Employed	16 (27.6)	0.8 (0.4–1.6)	0.542	1.45 (0.1–17.8)	0.761	2 (12.5)	0.3 (0.1–1.4)	0.160	NA	NA	9 (22.5)	0.6 (0.3–1.3)	0.214	3.4 (0.19–59.9)	0.376	1 (10.0)	0.2 (0.03–1.9)	0.182	NA	NA				
Education level																								
Never schooled	10 (17.2)	0.8 (0.4–1.7)	0.780	NA	NA	2 (12.5)	0.5 (0.1–2.5)	0.815	NA	NA	6 (15.0)	0.7 (0.3–1.7)	0.742	NA	NA	2 (20.0)	1.02 (0.2–4.9)	0.960	NA	NA				
Elementary	12 (20.7)	1.3 (0.6–2.7)	0.510	NA	NA	3 (18.8)	1.1 (0.2–3.9)	0.917	NA	NA	8 (20.0)	1.2 (0.5–2.8)	0.690	NA	NA	1 (10.0)	0.5 (0.1–4.1)	1.00	NA	NA				
High school	16 (27.6)	0.8 (0.4–1.5)	0.410	NA	NA	5 (31.2)	1 (0.3–2.9)	0.946	NA	NA	10 (25.0)	0.7 (0.3–1.4)	0.300	NA	NA	3 (30.0)	0.9 (0.2–3.6)	1.00	NA	NA				
University	20 (34.5)	Reference	-	NA	NA	6 (37.5)	Reference	-	NA	NA	16 (40.0)	Reference	-	NA	NA	4 (40.0)	Reference	0.498	NA	NA				
Numbers of sexual partners in life																								
One regular partner	52 (89.7)	Reference	-	NA	NA	14 (87.5)	Reference	-	NA	NA	34 (85.0)	Reference	-	NA	NA	9 (90.0)	Reference	-	NA	NA				
Several partners [1 to 5]	6 (10.3)	1.9 (0.8–4.8)	0.168	NA	NA	2 (12.5)	1.4 (0.3–6.3)	1.00	NA	NA	6 (15.0)	1.1 (0.4–2.9)	0.822	NA	NA	1 (10.0)	1.8 (0.2–14.4)	1.00	NA	NA				
Age at sexual onset (years)																								
< 16	17 (29.3)	0.9 (0.5–1.8)	0.890	NA	NA	3 (18.8)	0.5 (0.1–1.9)	0.405	NA	NA	12 (30.0)	1.0 (0.5–2.1)	0.995	NA	NA	2 (20.0)	0.6 (0.1–2.7)	0.728	NA	NA				

(Continued)

Table 2. (Continued)

Risk factors	Any HPV (N = 58)			Multiple HPV (N = 16)			HR-HPV (N = 40)			Multiple HR-HPV (N = 10)					
	n (%)	cOR (95%CI)	P*	aOR (95%CI)	P	n (%)	cOR (95%CI)	P*	aOR (95%CI)	P	n (%)	cOR (95%CI)	P*	aOR (95%CI)	P
16–20	32 (55.2)	Reference	-	NA	NA	10 (62.5)	Reference	-	NA	NA	21 (52.5)	Reference	-	NA	NA
> 20	9 (15.5)	1.2 (0.5–2.7)	0.672	NA	NA	3 (18.8)	1.5 (0.4–5.5)	0.471	NA	NA	7 (17.5)	1.4 (0.6–3.5)	0.464	NA	NA

*The P-value is calculated using Pearson's χ^2 test or Fisher's exact test for categorical variables;

**Not attributable ("NA") corresponds to variables giving crude Odds ratio not significant in univariate analysis ($P > 0.05$) and consequently not taken into account in multivariate analysis.

aOR: adjusted Odds ratio; cOR: crude Odds ratio; HIV: Human immunodeficiency virus; HR-HPV: High-risk human papillomavirus; LR-HPV: Low-risk human papillomavirus; NA: Not attributable; n: Number (size of study group); CI: Confidence interval.

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The variable “HIV infection” and the three subclasses of the variable “Occupation” (being unemployed, student or employed) were computed into the final multivariate analysis. Only the variables “HIV infection” succeeded to maintain its statistical link with the HPV outcomes variables. Indeed, HIV infection was strongly associated with cervical infection with any HPV (aOR: 17.4, 95% CI: 3.2–94.9%; $P = 0.001$), multiple types of HPV (aOR: 8.9, 95% CI: 2.0–39.6%; $P = 0.004$), HR-HPV (aOR: 13.2, 95% CI: 3.1–56.6%; $P < 0.001$) and cervical infection with multiple different HR-HPV (aOR: 8.4, 95% CI: 1.5–47.2%; $P = 0.015$). Finally, the other explicative variables taken into account in this analysis, such as “age at inclusion”, “marital status”, “education level”, “number of lifetime sexual partners” and “age at first intercourse” were found to be not significantly associated with any of the four outcomes variables characterizing cervical HPV infections in the study women.

Possible efficiencies of cervical HPV prevention by Gardasil vaccines

Finally, possible efficiencies of cervical HPV prevention in study women by the 4- and 9-valent Gardasil vaccines were further assessed. Less than 20% (12/58) of the HPV-positive cervical samples contained one of the 4 genotypes (HPV types 6, 11, 16 and 18) covered by the Gardasil-4 vaccine and less over (1.7%) contained simultaneously several of these 4-valent vaccine-genotypes. Regarding the Gardasil-9 vaccine, 50% (29/58) of HPV-positive cervical samples harbored at least 1 HPV type prevented by the 9-valent HPV vaccine, with 17.8% (5/28) of them which contained multiple HPV genotypes (Fig 2 and Table 1). Moreover, about 70% (27/40) of study women with a cervical HR-HPV infection harbored at least one 9-valent vaccine high-risk genotype. When excluding the non-vaccine type, half (50%; 20/40) of cervical HR-HPV infections were exclusively due to a 9-valent-vaccine high-risk genotype.

Discussion

The present study depicts for the first time the molecular epidemiology of cervical HPV infection in adult women living in N’Djamena, the capital city of Chad. One of five (22.9%) of study women had cervical HPV, which was mostly constituted of cervical HR-HPV (68.9%), 27.6% of HPV positive women harboring multiple genotypes. In this series, HIV seropositivity (3.5%) constituted the main risk factor significantly associated with an increasing risk of being infected by cervical HPV. The majority ($\approx 70\%$) of women infected by cervical HR-HPV showed high-risk genotypes covered by the 9-valent Gardasil-9 vaccine, with the HR-HPV-58 being the predominant genotype, followed by HPV-31, HPV-16, HPV-45, HPV-52 and HPV-18. Remarkably, most of these high-risk genotypes (HPV-58, HPV-31, HPV-45 and HPV-52) were not covered by the Gardasil-4 vaccine. Taken together, these observations highlight the unsuspected high burden of cervical HR-HPV infection in women more than 25 years living in Chad, with a high rate of preventable Gardasil-9 vaccine genotypes. These molecular findings demonstrate that cervical HR-HPV infection and associated risk for cervical cancer involve a large proportion of adult women living in Chad. According to our findings, with the hypothesis of a rate of genital shedding of HR-HPV at 15.8%, it may be estimated that at least 460,000 Chadian women aged more than 25 years thorough the country may be at risk for cervical cancer during their life. Finally, an *a priori* good predictive efficacy of the prophylactic Gardasil-9 vaccine could be envisioned for the primary prevention of cervical HPV infection in Chad. Thus, since HPV DNA molecular screening in Chad remains currently opportunistic or unavailable for many women, prophylactic HPV vaccination using a multivalent vaccine may help in achieving cervical cancer elimination.

In this large series of never screened adult women attending the obstetrics and gynecology clinic “*La Renaissance Plus*” of N’Djamena, we observed a high frequency (68.9%) of cervical

HR-HPV genotypes in HPV DNA positive women. Such high prevalence of HR-HPV appears notably higher as compared to those commonly reported in unscreened African women aged from 25 years of age and older, with cervical HR-HPV infection rates never exceeding half of the HPV-positive women. Indeed, cervical HR-HPV prevalences in adult women in sub-Saharan Africa vary frequently across regions of the same country and also from one country to another, and vary between 5.4% in Djibouti [36], 10.0% and 36.5% in Nigeria [17, 37], 12.5% in Democratic Republic of the Congo [38], 18.5% and 34.0% in Cameroon [39, 40], 19.3% in Malawi [41], 20.3% in Tanzania [42], 22.2% in Rwanda [43], 25.0% in South-Africa [19], 25.4% and 38.3% in Burkina Faso [44, 45], 39.3% in Madagascar [46] and finally 46.2% in Swaziland [47]. However, other studies conducted in other adult women living in sub-Saharan Africa reported high prevalences of cervical HR-HPV infection similar to that reported in the present series with HR-HPV prevalence, ranging from 60.4% in Nigeria [15], to 67.9% and 68.5% in South Africa [20, 48]. Only studies conducted in younger, but sexual active African women less than 25 years reported higher HR-HPV prevalences (70.0% to 84.0%) [18, 49–51] than that observed in our study. Different prevalences of cervical HPV in asymptomatic African women between studies, despite the fact that these studies were been conducted on the same continent and on quite similar populations, may be due to several factors, including firstly genital sampling methods, HPV molecular testing tools and behavioral and socio-demographic factors. On the other hand, the age could also constitute a major factor explaining the differences in HPV prevalences between studies, since the highest prevalence of HPV infection is found in the adolescence and early adulthood, soon after the sexual onset and then decline over time [7, 52].

Concerning HPV genotype distribution, women carrying HR-HPV infection showed remarkable high rates ($\approx 70\%$) of high-risk types targeted by the Gardasil-9 vaccine, with HPV-58 being the most represented genotype. Moreover, five other high-risk types included in the 9-valent vaccine (HPV-31, HPV-16, HPV-45, HPV-52 and HPV-18 in decreasing order) belonged to the four most detected genotypes; HPV-16 and HPV-18 being respectively the third and the fourth most represented. In the other hand, two high-risk types were not covered by the Gardasil-9 vaccine, HPV-35 and HPV-56.

Most previous studies conducted in sub-Saharan African countries depict a wide heterogeneity in the distribution of the main HR-HPV in women [14–20, 36, 38–48]. The predominance of cervical HR-HPV-58 genotype in Chadian women who never undergone cervical screening was never reported in other series from sub-Saharan Africa, and may constitute a particularity of the molecular epidemiology of HR-HPV in women living in Chad. Our observations furthermore demonstrate the preponderance of cervical HR-HPV targeted by the Gardasil-9 vaccine in asymptomatic women living in Chad, as commonly observed in women with normal cytology and those with high-grade intraepithelial lesions in other sub-Saharan African countries [14]. These indications are of paramount importance because they allow evaluating the predictive efficiency of vaccination against HPV with the Gardasil-9 vaccine in young Chadian girls. Indeed, our observations suggest that a large majority ($\approx 70\%$) of these HR-HPV infections could have been, *a priori*, prevented using the Gardasil-9 vaccine. In the context of the important population of females living in Chad, estimated in 2018 at around 7,600,000 [22], our epidemiological observations represent a powerful argument in favor of the introduction of the Gardasil-9 vaccine in the national immunization program in order to strengthen cervical cancer prevention.

In the present series, two main groups characterized by their risk factors for HPV infection were identified. Firstly, HIV-infected women were found to be 17-times more at risk for cervical infection with any type of HPV and more than 8-times for multiple types of HPV than HIV-negative women. Regarding cervical HR-HPV infection, HIV-infected women were

13-times more at risk than HIV-negative women and more than 8-times for infection with multiple high-risk types. These findings are consistent with previous reports demonstrating the aggravating effect of HIV in the burden of HR-HPV infection in women and particularly in the African settings [53–55]. Secondly, the student women, being mostly 25 to 29 year-old, were more than 2 times more at-risk for both cervical infection with any type of HPV and HR-HPV infection than women involved in other social occupations. However, this risk seems to fade in multivariate analysis. In our study, we did not find by multivariate analysis any association between any HPV and more specifically HR-HPV genital carriage and young age, although age less than 30 years was previously reported as a risk factor frequently associated with cervical HR-HPV infection in African women [15, 42, 50]. The possibility exists that our study may have lacked of power due to too small sample size of young women group to evidence such association. Other factors such as genital toilet or hygiene practice during menses which modify the cervicovaginal ecology [56, 57], or the higher economic status of student women which constitutes per se a multiple risk behavior [58], could be also envisioned.

Our observations highlight that women living in Chad constitute a neglected high risk group for cervical HR-HPV infection and consequently for cervical cancer. These findings constitute the first report ever provided on the epidemiological burden of HPV infection in Chad. The very high prevalence of cervical HR-HPV in adult women clearly demonstrates that cervical HR-HPV infection in Chad constitutes a major public health problem which remains largely unsuspected. Therefore, there is an urgent need for implementing a cervical cancer prevention program in Chad, as recommended by the WHO [59]. According to Mortier and colleagues, the cytology-based cervical cancer screening in women in Chad is feasible with low cost and easy to interpret visual technics; and could be integrated in existing healthcare structures [25]. Indeed, for these women carrying cervical HR-HPV infection, only secondary prevention with regular screening for precancerous lesions by cytology and the monitoring of the viral persistence by HPV molecular testing, remains the only alternative to prevent the disease progression into invasive cervical cancer. However, in the context of Chad, a very low-income country, there is a serious lack of pathologist specialist thereby making conventional cytology not suitable and reinforcing on the other hand the great necessity to implement HPV DNA testing with molecular technologies, including point-of-care analyzers [7]. Indeed, HPV DNA testing is an excellent alternative to cytology for cervical cancer screening, because it is higher sensitive, more reproducible and easy to interpret than cytology [7]. Moreover, the rapid turn-around time of HPV DNA testing system could promote the “see and treat” approach recommended by the WHO [59], thus allowing to maximize all the medical support in a single visit and avoiding the loss of women positive for HR-HPV. Furthermore, taking into account that most adult Chadian women are living in remote rural areas, far away from adequate healthcare structure, self-collection cervical specimen carried out at home by women themselves could also represent a relevant alternative allowing to increase the coverage of screening when coupled with low-cost HPV DNA testing technologies [7]. This model of multicomponent prevention strategy that could also integrate existing HIV healthcare structures is critical to better target adult women living in Chad at risk for cervical HR-HPV infection and related diseases. However, without medical insurance, as it is the case for most women in Chad, secondary prevention based on HPV DNA molecular diagnostic tests is still beyond the reach of most Chadian women. Consequently, along with the secondary prevention strategy adapted to Chadian adult women, around 600,000 adolescents girls aged from 10 to 14 year-old in Chad [22, 23], could benefit from a national immunization program with the current prophylactic Gardasil-9 vaccine prior their sexual life onset [7].

We tried to limit the selection bias of the study population in order to make this survey as much representative as possible of the female population in Chad. Thus, the study inclusions

were initially carried out by random sampling of districts in N'Djamena, with further selection of neighborhoods inclusions site. Study women were referred to the clinic "La Renaissance Plus" of N'Djamena, although they were not part of the patients from the clinic, avoiding the obvious bias of recruitment by health care facilities. Furthermore, 9 (3.5%), 19 (7.5%) and 8 (3.2%) study participants were HIV-, HBsAg- and HCV-specific antibody- positive, respectively, in accordance with the high endemicity of these three major chronic viral infections in Chad [60, 61]. Indeed, Chad is a country of generalized HIV epidemic with the adult prevalence rate at 3.5% in N'Djamena [60], as observed in our series. Chad is also a country of high HBV and HCV infections prevalences in adults, and in our study the prevalences of these viral infections were of the same order as those previously reported in N'Djamena in 2014 by Bessimbaye and colleagues [61]. Consequently, the rates of these three epidemiological infectious markers observed in our series tend to reflect the general epidemiology in Chad, thereby making our study sample close to the general population in Chad. However, our study has some limitations. First, the representativeness of the included study population is not completely ensured for the other districts of N'Djamena which were not selected. Such inclusion bias could be extended in other cities and rural remote territories of Chad, where no women could be included. Second, the small sample size of our study population may have introduced a bias, although more than 250 women were enrolled. Third, participants were included on a voluntary basis. This latter approach may be a source of recruitment bias. Furthermore, by using a face-to-face questionnaire, the validity of the answers to the questions was collected from participants, including items related to the intimacy of their sexual life. In Chad, a high number of women are unable to read and write properly, rendering a self-administered questionnaire was not relevant for this reason; however, it has been documented that questions about personal privacy are best collected anonymously by means of a self-administered questionnaire [62]. In addition, the recruitment based on community-churches, and associative networks may have introduced a selection bias. Moreover, the study women said that they agreed to participate in the study because they considered that confidentiality was guaranteed at the clinic "La Renaissance Plus" of N'Djamena, which is considered by the public as the benchmark of good practice in the area of obstetrics and gynecology. Finally, in regard of our small sample size some risk factors may have been underestimated in the statistical analyses. Despite these possible limitations, our study constitutes the first report providing objective information on molecular epidemiology of cervical HPV in symptom-free adult women living in N'Djamena, and thus enables us to show all the interest of the prophylactic vaccination by the Gardasil-9 vaccine in eligible young adolescent women, that could be very possibly generalized to the whole country.

In conclusion, an unsuspected high prevalence of cervical HR-HPV infection, worsened by HIV, was observed in women more than 25 years living in N'Djamena, Chad. The study women recruitment according to a population-based approach allows to extrapolate our observations to the general female population, and to make the hypothesis that the burden of HR-HPV shedding may be particularly high in Chadian women, and consequently, the risk of further development of HPV-associated cervical precancerous and neoplastic lesions. In addition, the HPV genotypes distribution in genital secretions of study women indicates the probable existence of a particular molecular epidemiology of HPV in Chad, with rare genotypes being predominant such as HPV-58. Finally, our results demonstrate that the majority of cervical HR-HPV corresponds to preventable Gardasil-9 vaccine genotypes, making the rationale of introducing primary prevention against cervical cancer in young female adolescents living in Chad by prophylactic vaccination. Taken together, our findings point for the first time the unsuspected high exposure of Chadian women to oncogenic HR-HPV, making thus cervical cancer, its diagnosis and prevention, some of the most important public health challenges that Chad will faced in a near future.

Supporting information

S1 File. Baseline database of the 253 study women living in N'Djamena, Chad. Microsoft excel baseline database containing all information from the study women analyzed in the study and related to their sociodemographic and clinical characteristics and also information associated to the results of the HPV testing and genotyping.
(XLSX)

S2 File. STROBE Checklist.
(DOC)

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2. Forte prévalence de l'infection cervicale à Papillomavirus humains à haut-risque, causée par des génotypes atypiques, au sein d'une population de femmes adultes, immigrées de première génération, originaires d'Afrique sub-saharienne et vivant en France.

Ce paragraphe est principalement basé sur l'article intitulé "*High prevalence of cervical high-risk human papillomavirus harboring atypical genotypes in first-generation adult immigrant women originating from sub-Saharan Africa and living in France*", soumis pour publication dans la revue scientifique "*Journal of Immigrant and Minority Health*" et actuellement en cours de révision en date du 04 septembre 2019. Il traite de l'épidémiologie de l'infection à papillomavirus humains (HPV) et les principaux facteurs de risque associés, au sein d'une population de femmes adultes immigrées originaires d'Afrique sub-Saharienne et vivant en France.

Avant-propos.

Rationnel et objectif de l'étude. Environ 300.000 femmes immigrées originaires d'Afrique subsaharienne vivent en France (INSEE 2014). La plupart des immigrées africaines de première génération arrivent en France au début de leur vie adulte et la plupart d'entre elles arrivent déjà sexuellement actives (INSEE 2014). Les femmes africaines immigrées de première génération vivant en France pourraient présenter un profil infectieux reflétant l'épidémiologie de leur pays d'origine, où l'infection cervicale à HR-HPV est très répandue et exacerbée par la synergie dite « *syndémique* » jouée par l'épidémie de VIH et d'autres infections sexuellement transmissibles (IST) (McCloskey et al., 2017). L'objectif de la présente étude transversale était d'évaluer la prévalence de l'infection cervicale à HR-HPV, la distribution des principaux génotypes impliqués ainsi que les facteurs de risque associés, chez des femmes africaines immigrées séropositives et séronégatives vivant en France et fréquentant le Centre Hospitalier Régional d'Orléans, France.

Méthodologie. Les femmes africaines immigrées de première génération fréquentant le Centre Hospitalier Régional d'Orléans (France) ont été prospectivement incluses dans l'étude. La détection de l'infection cervicale à HPV a été réalisée à partir d'écouvillons endocervicaux en utilisant le système de PCR en temps réel multiplex Anyplex II HPV28 genotyping test (Seegene, Seoul, Corée du Sud). Les femmes incluses dans l'étude ont également été soumises au frottis endocervical pour le dépistage de lésions précancéreuses par cytologie.

Résultats. Cinquante femmes (âge moyen : 41,7 ans) vivant en France (durée moyenne de séjour : 10,7 ans) ont été incluses. La prévalence du VIH était élevée (74,0%). La prévalence

de l'infection cervicale à HPV était de 68,0%, avec 56,0% de HR-HPV. Les génotypes HR-HPV-68 et -58 étaient les génotypes prédominants (20,0% et 14,0%, respectivement). Les génotypes HR-HPV-16 et HR-HPV-18 étaient par contre rarement détectés. La plupart des femmes (84,0%) présentaient une cytologie normale, tandis que les autres (16,0%) présentaient des anomalies cervicales et étaient fréquemment infectées par le VIH (87,5%).

Conclusion. Ces observations mettent en évidence le lourd et insoupçonné fardeau que représente les infections cervicales à HR-HPV associées principalement à des génotypes atypiques, à une infection par le VIH et à des anomalies cervicales chez des femmes africaines immigrées de la première génération vivant en France.

High prevalence of cervical high-risk human papillomavirus harboring atypical genotypes in first-generation adult immigrant women originating from sub-Saharan Africa and living in France

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Running head. Cervical oncogenic HPV in immigrant African women in France

Abstract

Background. Human papillomavirus (HPV)-related cervical lesions in first-generation immigrant African women in France should reflect the epidemiology of high-risk (HR)-human papillomavirus (HPV) infection in sub-Saharan Africa.

Methods. First-generation immigrant African women attending the Centre Hospitalier Régional of Orléans, France, were prospectively subjected to endocervical swabs for HPV DNA PCR and Pap smear.

Results. Fifty women (mean age, 41.7 years) living in France (mean stay, 10.7 years) were included. Prevalence of HIV was high (74.0%). Cervical HPV prevalence was 68.0%, with 56.0% of HR-HPV. HR-HPV -68 and -58 were the predominant genotypes (20.0% and 14.0%, respectively). HR-HPV-16 and HR-HPV-18 were infrequently detected. Most women (84.0%) showed normal cytology, while the remaining (16.0%) exhibited cervical abnormalities and were frequently HIV-infected (87.5%).

Conclusion. These observations highlight the unsuspected high burden of cervical HR-HPV infections mostly associated with atypical genotypes, HIV infection and cervical abnormalities in first-generation immigrant African women living in France.

Keywords. Human papillomavirus; First-generation; Immigrates Women; Sub-Saharan Africa; France; Human immunodeficiency virus; Cervical cancer.

Background

Human papillomavirus (HPV) infection is the most common viral sexually transmitted infection (STI) worldwide and high-risk (HR)-HPV genotypes are responsible for 5.2% of all cancers worldwide, 2.2% of cancers in developed countries and 7.7% of all cancers in developing countries (1-3). In sub-Saharan Africa, cervical cancer associated with persistent cervical HR-HPV infection is the most common cancer in women in many countries, with more than 75,000 new cases and nearly 50,000 deaths occurring each year (4, 5). According to the World Health Organization, cervical cancer will kill annually about half of a million women by the next decade, mostly in Sub-Saharan Africa where HIV epidemic and other risk factors are worsening the burden of this cancer (5,6). Thus, cervical cancer has become progressively one of the main public health challenges to overcome in sub-Saharan Africa since the recent decades (7).

Sub-Saharan African adult female population harbors high rates of cervical HR-HPV infections that can reach 46.2% in HIV-negative and 79.1% in HIV-positive women, with frequent variations from one region to another (8-10). Likewise, there is a wide heterogeneity in the distribution of circulating HR-HPV genotypes throughout the African continent (10). The high-risk genotypes targeted by the Gardasil-9® vaccine (HPV-16, -18, -31, -33, -45, -52, -58), and also non-vaccine high-risk types such as HPV-35, HPV-68 and HPV-59 are among the most detected genotypes both in African women with normal cytology as well as in those with low and high-grade cervical lesions and cancers (8-11).

Around 300,000 immigrant women originating from sub-Saharan Africa are living in France (12). Most of first-generation African immigrant women arrive in France at the beginning of their adult life and most of them arrive already sexually active (12). First-generation immigrant African women living in France could harbor an infectious profile reflecting the epidemiology of their country of origin where cervical HR-HPV infection is highly prevalent and exacerbated by the so-called “syndemic” synergy played by HIV epidemic and other sexually transmitted infections (STIs) (13).

The aim of the present cross-sectional study was to assess the prevalence and genotypes distribution of cervical HR-HPV infection and associated risk factors in HIV-positive and HIV-negative immigrant African women living in France and attending the Centre Hospitalier Régional of Orléans, France.

Methods

Study design. The study was a descriptive cross-sectional survey to assess molecular testing for cervical HPV, cervical cytology and HIV infection among first-generation immigrant African women (at least 25 years) attending the Centre Hospitalier Régional d'Orléans.

Enrolment, selection criteria and study population. First-generation immigrant African women attending the Centre Gratuit d'Information, de Dépistage et de Diagnostic (CeGIDD) of the Centre Hospitalier Régional d'Orléans were prospectively included in 2018. The CeGIDD is an outpatient consultation service providing HIV, STI hepatitis and HPV prevention, screening and care for the general adult population.

The inclusion criteria were being originating from sub-Saharan Africa, having given signed informed consent, being aged between 25-65 years, being sexually active, having no genital troubles at physical examination and having completed the study questionnaire. Exclusion criteria included age less than 25 years and more than 65 years, not willing to participate to the study or to answer the face-to-face sociodemographic and medical data questionnaire.

After signed the informed consent form, the included women benefited from free STIs screening including HIV, Hepatitis B (HBV) and C (HCV) viruses, Chlamydia trachomatis, Neisseria gonorrhoeae, genital herpes and Trichomonas vaginalis. All women received an information session on HIV, STIs and cervical cancer, and completed a face-to-face questionnaire that included socio-demographic characteristics and behavioral data such as age, marital status, social occupation, education level, history of STI, HIV status and also sexual behavioral characteristics such as the number of lifetime sexual partners, frequency of condom use and the age at first sexual intercourse. Women diagnosed positive for any STI received adequate care and those negative for HBV were proposed to be vaccinated against HBV.

Samples and processing. After completing the medical and socio-demographic data collection questionnaire, a nurse performed cervicovaginal sampling using a flocked swab (Copan Diagnostic Inc., California, USA), frozen at -80°C. Cervicovaginal swab were transported in frozen ice packs, to the virology laboratory of the Hôpital européen Georges Pompidou, Paris, France, for molecular analyses.

After the sample procedure, included women were referred to the gynecologist to undergo Pap smear cytology. Cytological results were classified according to the Bethesda

system 2001 (14). Samples were classified as atypical squamous cell of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSILs), high-grade squamous intraepithelial lesions (HSILs), adenocarcinoma in situ (AIS) and carcinoma. Cytology was evaluated by cytoscreeners and pathologists blinded to the outcomes of HPV testing.

HPV detection and genotyping. HPV detection and genotyping were assessed using the real-time PCR assay Anyplex™ II HPV28 (Seegene, Seoul, South Korea) as described previously (3).

Statistical analyses. Means and standard deviations (SD) were calculated for quantitative variables and proportions for categorical variables. The results were presented as a 95% confidence interval (CI). Pearson's χ^2 or Fisher's exact tests were used for categorical variables and the non-parametric Mann-Whitney U-test for quantitative variables.

Ethics statement. The Scientific Committee of the Centre Hospitalier Régional d'Orléans formally approved the study. All included women gave their informed signed consent to participate to the study. For each included woman, the record of the consent to participate to the study was documented on each questionnaire. The Ethical Committee formally approved this consent procedure. Note also that a feedback of medical results and particularly all results of HPV detection and genotyping were given to each participant. Women infected with any STI, those harboring cervical HR-HPV and women with HPV-related lesions received adequate treatment and therapeutic follow up.

Results

Characteristics of study participants. A total of 50 women (mean age, 41.7 years; range, 25-65) living in Orléans, France, for an average of 10.7 years (range, 1-32) was prospectively included (Table 1). The study population was mainly constituted by HIV-infected women (37/50; 74.0%) and 26.0% (13/50) were HIV-negative.

Table 1. Baseline characteristics of 50 African immigrant women living in Orléans, France.

Characteristics	Study women (N=50) n (%) [95% CI]*
Age	
All age [mean (SD), years]	41.7 (10.3)
25-29	5 (10.0) [1.7-18.3]
30-39	18 (36.0) [22.7-49.3]
40-49	18 (36.0) [22.7-49.3]
≥ 50	9 (18.0) [7.3-28.6]
HIV infection	37 (74.0) [61.8-86.2]
Marital status	
Single	23 (46.0) [32.2-59.8]
Living in couple	19 (38.0) [24.5-51.4]
Divorced	7 (14.0) [4.3-23.6]
Widowed	1 (2.0) [0.0-5.8]
Times since the entry in France (Years)	
< 5	15 (30.0) [17.3-42.7]
5-10	11 (22.0) [10.5-33.5]
11-20	20 (40.0) [26.4-53.6]
> 20	4 (8.0) [0.5-15.5]
Occupation	
Student	3 (6.0) [0.0-12.6]
Unemployed	29 (58.0) [44.3-71.7]
Employed	18 (36.0) [22.7-49.3]
Sexual behaviors	
Age at sexual onset [years]	
< 16	6 (12.0) [2.9-21.1]
16-20	37 (74.0) [61.8-86.2]
> 20	7 (14.0) [4.3-23.6]
Number of sexual partners in the last 12 months	
No partner	15 (30.0) [17.3-42.7]
One regular partner	34 (68.0) [55.1-80.9]
Several partners [1 to 5]	1 (2.0) [0.0-5.8]
Frequency of condom use	
Always	6 (12.0) [2.9-21.1]
Inconsistently	30 (60.0) [46.4-73.6]
Never	14 (28.0) [15.5-40.4]
Cytological cervical results	
Normal cytology	42 (84.0) [73.8-94.2]
Low grade lesion	3 (6.0) [0.0-12.6]
High grade lesion	2 (4.0) [0.0-9.4]
ASCUS	3 (6.0) [0.0-12.6]
HPV DNA detection and types	
HPV DNA in swab	34 (68.0) [55.1-80.9]
Multiple types of any HPV	12 (24.0) [12.2-35.8]
LR-HPV	15 (30.0) [17.3-42.7]
Possibly oncogenic HPV	4 (8.0) [0.5-15.5]
HR-HPV	28 (56.0) [42.2-69.7]
Multiple types of HR-HPV	7 (14.0) [4.3-23.6]
Any 9-valent vaccine types**	20 (40.0) [26.4-53.6]
Multiple 9-valent vaccine types	3 (6.0) [0.0-12.6]
9-valent vaccine HR-HPV types	19 (38.0) [24.5-51.4]
9-valent vaccine HR-HPV types only	12 (24.0) [12.2-35.8]
Non-vaccine HR-HPV types only	10 (20.0) [8.9-31.1]

* The frequency of each variable is presented with their 95% confidence interval in brackets;

**The 9-valent Gardasil-9® vaccine (Merck & Co. Inc.) is effective against HPV genotypes 6, 11, 16, 18, 31, 33, 45, 52 and 58.

95% CI: 95% confidence interval; HIV: Human immunodeficiency virus; HPV: Human papillomavirus; LR-HPV: Low-risk human papillomavirus; HR-HPV: High-risk human papillomavirus.

Single women (46.0%) were a little more represented than women living in life-couple with a male partner (38.0%). Although the majority of these African immigrant women were unemployed (58.0%), a relatively high number of them (36.0%) reported to have a regular paid job in France, while only 3 women (6.0%) were students. For most of the women (74.0%) sexual intercourse onset occurred between 16 to 20 years. The majority (68.0%) having had sexual intercourse with one regular partner during the last 12 months. Unprotected sexual intercourse was particularly frequent, as only 12.0% of study participants declared to always use systematically condom during sex, while the large majority (60.0%) of them reported to use condom inconsistently during sex. Unexpectedly, 28.0% of participants declared to categorically never use condom with their sexual partner.

Finally, two (4.0%) women declared having had previous acute genital herpes and another one (2.0%) reported past history of genital chlamydia. No past history of any other STIs such as syphilis, gonorrhoea and genital condyloma was declared. In addition, no women were vaccinated against HPV infection and almost all of them had never undergone cervical Pap smear before the study period. Furthermore, all HIV-negative women reported to have never been screened for HPV-related cervical lesions.

The Table 2 depicts the characteristics of study women according to HIV serostatus. HIV-positive women reported more frequently to never use condom during sex than HIV-negative women ($P < 0.05$). None of the students included in this study were HIV-infected despite the fact that all of them reported to use condom only occasionally. Finally, no other statistically significant difference could be found between HIV-infected and HIV-uninfected women in this study.

Table 2. Characteristics of the study women according to their HIV serostatus.

	HIV-positive women (N=37) n (%) [95% CI]*	HIV-negative women (N=13) n (%) [95% CI]*	P**
Characteristics			
Age			
All age [mean (SD), years]	42.8 (9.7)	38.6 (11.6)	0.17
25-29	2 (5.4) [0.0-12.7]	3 (23.1) [0.2-45.9]	0.10
30-39	13 (35.1) [19.7-50.5]	5 (38.4) [12.1-64.9]	1
40-49	16 (43.2) [27.3-59.2]	2 (15.4) [0.0-35.0]	0.09
≥ 50	6 (16.2) [4.3-28.1]	3 (23.1) [0.2-45.9]	1
Marital status			
Single	14 (37.8) [22.2-53.5]	9 (69.2) [44.1-94.3]	0.06
Living in couple	16 (43.2) [27.3-59.2]	3 (23.1) [0.2-45.9]	0.32
Divorced	6 (16.2) [4.3-28.1]	1 (7.7) [0.0-22.2]	0.65
Widowed	1 (2.7) [0.0-7.9]	0 (0.0) [0.0-0.0]	1
Times since the entry in France (Years)			
< 5	10 (27.1) [12.7-41.3]	5 (38.4) [12.1-64.9]	0.49
5-10	8 (21.6) [8.4-34.9]	3 (23.1) [0.2-45.9]	1
11-20	16 (43.2) [27.3-59.2]	4 (30.7) [5.7-55.8]	0.52
> 20	3 (8.1) [0.0-16.9]	1 (7.7) [0.0-22.2]	1
Occupation			
Student	0 (0.0) [0.0-0.0]	3 (23.1) [0.2-45.9]	0.014
Unemployed	21 (56.7) [40.8-72.7]	8 (61.5) [35.1-87.9]	1
Employed	16 (43.2) [27.3-59.2]	2 (15.4) [0.0-35.0]	0.09
Sexual behaviors			
Age at sexual onset [years]			
< 16	6 (16.2) [4.3-28.1]	0 (0.0) [0.0-0.0]	0.32
16-20	28 (75.7) [61.8-89.5]	9 (69.2) [44.1-94.3]	0.72
> 20	3 (8.1) [0.0-16.9]	4 (30.7) [5.7-55.8]	0.06
Number of sexual partners in the last 12 months			
No partner	12 (32.4) [17.3-47.5]	3 (23.1) [0.2-45.9]	0.73
One regular partner	24 (64.8) [49.5-80.2]	10 (76.9) [54.1-99.8]	0.51
Several partners [1 to 5]	2 (5.4) [0.0-12.7]	0 (0.0) [0.0-0.0]	1
Frequency of condom use			
Always	6 (16.2) [4.3-28.1]	0 (0.0) [0.0-0.0]	0.32
Inconsistently	17 (45.9) [29.8-62.0]	13 (100.0) [100.0-100.0]	0.0005
Never	14 (37.8) [22.2-53.5]	0 (0.0) [0.0-0.0]	0.01
Cytological cervical results			
Normal cytology	30 (81.1) [68.4-93.7]	12 (92.3) [77.8-100.0]	0.66
Low grade lesion	3 (8.1) [0.0-16.9]	0 (0.0) [0.0-0.0]	0.55
High grade lesion	2 (5.4) [0.0-12.7]	0 (0.0) [0.0-0.0]	1
ASCUS	2 (5.4) [0.0-12.7]	1 (7.7) [0.0-22.2]	1
HPV DNA detection and types			
HPV DNA in swab	26 (70.3) [55.5-85.0]	8 (61.5) [35.1-87.9]	0.73
Multiple types of any HPV	11 (29.7) [15.0-44.4]	1 (7.7) [0.0-22.2]	0.14
LR-HPV	13 (35.1) [19.7-50.5]	2 (15.4) [0.0-35.0]	0.29
Possibly oncogenic HPV	4 (10.8) [0.8-20.8]	0 (0.0) [0.0-0.0]	0.56
HR-HPV	23 (62.2) [46.5-77.8]	5 (38.4) [12.1-64.9]	0.19
Multiple types of HR-HPV	6 (16.2) [4.3-28.1]	1 (7.7) [0.0-22.2]	0.66
Any 9-valent vaccine types**	16 (43.2) [27.3-59.2]	4 (30.7) [5.7-55.8]	0.52
Multiple 9-valent vaccine types	3 (8.1) [0.0-16.9]	0 (0.0) [0.0-0.0]	0.55
9-valent vaccine HR-HPV types	16 (43.2) [27.3-59.2]	3 (23.1) [0.2-45.9]	0.32
9-valent vaccine HR-HPV types only	11 (29.7) [15.0-44.4]	1 (7.7) [0.0-22.2]	0.14
Non-vaccine HR-HPV types only	6 (16.2) [4.3-28.1]	4 (30.7) [5.7-55.8]	0.42

Prevalence of HPV detection, genotype distribution and cytological results. Overall, more than two-thirds of study women were shedding genital HPV DNA, corresponding to a total HPV prevalence of 68.0% (34/50), with 56.0% (28/50) carrying HR-HPV genotypes (Table 1). Genital infection profiles with multiple HPV genotypes were relatively frequent (24.0%), with an average of 2.1 HR-HPV (range, 1 to 4) per cervical swab sample.

HIV-infected women showed a trend to be more frequently infected by HPV than HIV-negative women, but without statistical significance (70.3% versus 61.5%; P=0.73). Similarly, HIV-infected women were almost twice more infected by HR-HPV than HIV-negative women, but the difference was not significant (62.2% versus 38.4%; P=0.19).

The Figure 1 depicts the atypical profile in the distribution of HPV genotypes in HPV-DNA positive cervical samples, with the non-vaccine HR-HPV type 68 (20.0%) being the predominant genotype, followed by the Gardasil-9® vaccine HR-HPV type 58 with a prevalence of 14.0%. HR-HPV-16 and HR-HPV-18 were respectively detected in 6.0% and 10.0% of cervical swabs. Apart from the HPV-31 found in 8.0% of the women, the other Gardasil-9® vaccine types (HPV-6, HPV-11, HPV-33, HPV-45 and HPV-52) were each detected only in one (2.0%) woman. Similarly, the other non-vaccine HR-HPV types (HPV-35, HPV-39, HPV-56 and HPV-59) were also represented with very low proportion.

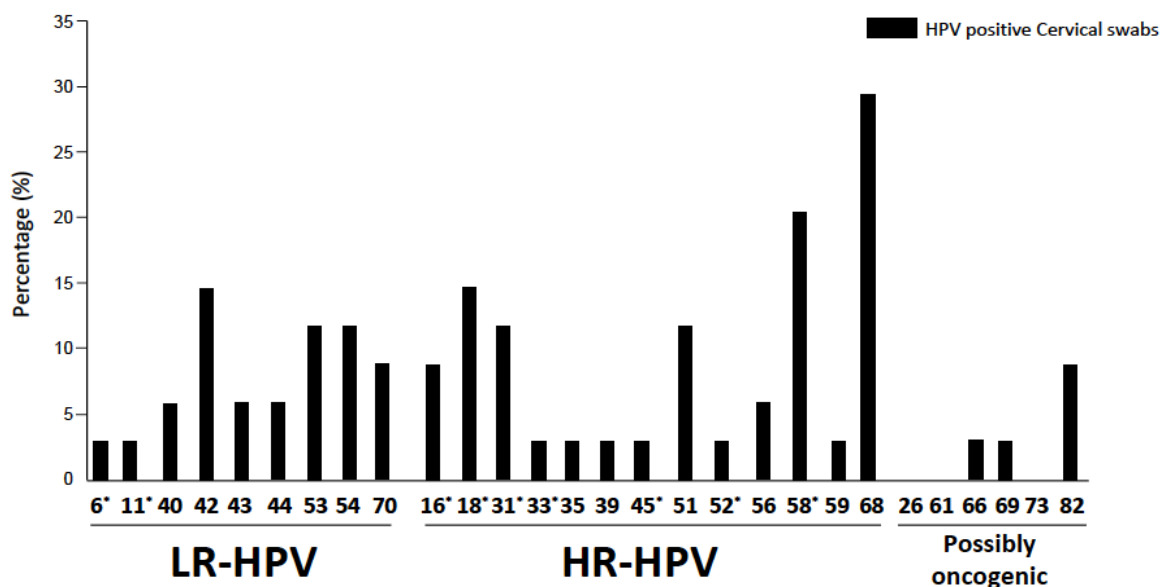


Figure 1. Proportion of cervical HPV genotypes according to their inclusion in the 9-valent Gardasil-9® vaccine. Number of low risk (LR), high risk (HR) and possibly oncogenic HPV genotypes in 34 cervical specimens positives for HPV DNA by molecular biology according to their possible prevention by 9-valent Gardasil-9® HPV vaccine among adult first-generation immigrant women originating from sub-Saharan Africa and living in France. According to the HPV classification

nomenclature provided by the International Agency for Research on Cancer (IARC) [Muñoz et al., 2003; Muñoz et al., 2006, Bouvard et al., 2009], Anyplex™ II HPV28 allows to distinguish 28 HPV genotypes, including 13 high-risk types (HR-HPV -16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68), 9 low-risk (LR) types (LR-HPV -6, -11, -40, -42, -43, -44, -53, -54 and -70) and then, 6 genotypes classified as possibly carcinogenic (HPV-26, -61, -66, -69, -73 and -82). Study women were mainly originating from Central Africa, including Democratic Republic of Congo (32.0%), Cameroon (14.0%), Central African Republic (10.0%), Angola (6.0%), Rwanda (6.0%), Burundi (2%) and Gabon (2%), while the remaining women were from West Africa, including Ivory Coast (10.0%), Nigeria (4%), and other west-African countries (Guinea, Mali, Mauritania, Senegal and Togo; 2%). One (2.0%) participant was from Ethiopia.

Nota bene. The 9-valent Gardasil-9® vaccine (Merck & Co. Inc., New Jersey, USA) is effective against HPV genotypes -6, -11, -16, -18, -31, -33, -45, -52 and -58, as indicated by stars.

Results of Pap smear cytological exam are shown in the Table 1. The large majority of women (84.0%) showed normal cytology, but 6.0% harbored low-grade lesions and ASCUS, and only two women (4.0%) exhibited high-grade cervical lesions. There was no difference in the Pap smear interpretation results between HIV-infected and HIV-negative women (Table 2).

Finally, no predictive risk factor for cervical HPV infection in these women has could be evidenced in univariate and multivariate analyses.

Discussion

We herein report unsuspected high burden of cervical HR-HPV infection in a consecutive series of non-vaccinated first-generation adult immigrant women originating from sub-Saharan Africa and living in France. Genital shedding of HPV and HR-HPV were highly frequent in these African immigrant women. Unexpectedly, the non-vaccine HPV-68, a genotype rarely found within the French female population, was the most frequently detected. These findings point the remarkable and atypical epidemiological profile of cervical HPV infection in first-generation African immigrants living in France, with a rare and non-vaccine high-risk genotype being the most frequently detected. Only two-fifth of the cervical HR-HPV infections observed in the study could have been a priori prevented by the prophylactic Gardasil-9® vaccine, raising the concern about the low predictive efficacy of prophylactic HPV vaccine in the population of African immigrants residing in France.

In these African immigrant women, we found a particularly high prevalence of cervical HPV (68.0%), frequently associated with HR-HPV genotypes (56.0%) found in 61.5% of HIV-negative and 70.3% of HIV-positive women. Such high prevalences of cervical HR-HPV in African emigrates appear uncommon and higher than that HPV prevalences previously reported in the French female general population with cervical HR-HPV infection rates oscillating from 8% to 22.8% in HIV-negative adult women with normal cytology (15-24) and 26.4% in HIV-positive women (25). A large population-based survey on HIV-

positive women, including six of the main European capital cities, reported cervical HR-HPV prevalences around 35.0% (26), much lower than that found in our study population. On the other hand, the high prevalences of HR-HPV infection found in the present study mirror the burden of cervical HR-HPV infection commonly reported in Sub-Saharan Africa. Indeed, quite similar to our study, cervical HR-HPV prevalences in sub-Saharan Africa can reach 46.2% in HIV-negative adult women (more than 25 years) and 79.1% in HIV-positive women (8-10). These observations highlight that immigrant women originating from sub-Saharan Africa and living in France, irrespectively of their HIV-status, are at least twice more infected by HR-HPV than both the French and European female population. As a consequence, adult first-generation African immigrant women living in France, especially those infected with HIV, should be at very high risk for developing cervical cancer. Further multicenter studies with larger sample size are now needed to better estimate the cervical cancer burden in the population of first-generation immigrant women originating from sub-Saharan Africa and living in France. In addition, secondary prevention with regular screening for precancerous lesions by cytology should be strengthened and even improved with new social sensitization strategies in order to reach this specific subgroup of the female population of France.

The distribution of HPV genotypes in women, with a majority harboring the non-vaccine HPV-68, was different to that previously reported in French women born to French parents with HR-HPV-16 as the most detected genotype (22). This unusual and original epidemiological profile of HPV infection in a subgroup of the French population could be explained by the social conditions of these immigrant women at their arrival in France. Indeed, immigrant women from sub-Saharan Africa living in France, especially those of first-generation, remain mostly in the same social network at their arrival in France, which is mainly constituted by people also coming from their native regions where these unusual genotypes are frequent (27).

The wide heterogeneity of the HPV genotypes distribution in our study female population was previously reported in Africa (7, 8, 10, 11). As for HIV epidemic in some countries, in which imported sub-epidemics may occur, characterized by HIV-subtypes and strains genetically and phenotypically different than those commonly observed (28), adult immigrant women originating from sub-Saharan Africa could also import HPV genotypes different from those commonly spreading in French women born in France. Such imported viruses could be more aggressive and phenotypically less recognizable by the immune system of vaccinated people (29). Further genetic studies are obviously needed to better address this aspect of HPV epidemiology.

In France prophylactic vaccination against HPV infection is currently recommended for very young girls (11-14 years), with a catch up until 19 years, preferably before the onset of sexual activity in order to prevent any infection before the first exposures (30). Interestingly, the Gardasil-9® vaccine could theoretically prevent only two-fifth of HPV infections in the study population, a feature that however appears quite impossible to reach because of early sex past-history of study women before their arrival in France.

Finally, in our series of first-generation African immigrant women living in France and who undergone cervical cancer screening for the first time, we found a large proportion (84.0%) of normal cytological results, and only a minority exhibited pathological Pap smear test, including ASCUS (6.0%) observed both in one HIV-infected and one HIV-negative woman, and also low-grade (6.0%) and high-grade (4.0%) cervical lesions only observed in HIV-positive women. Although there was no statistically difference between HIV-infected and HIV-negative women in regards to Pap smear results, our findings show that worst cervical lesions are mostly found in HIV-positive women than in HIV-negative. Further large studies are needed to have a more concrete landscape on the burden of cervical lesions and cancers in the African immigrant female population living in France in which the coverage of cervical cancer screening remains very low. Indeed, in France, foreign women born to parents coming from a foreign country were above 5 times more likely to have never been screened for cervical cancer in their lifetime than French women born to French parents (31). Furthermore, recent population-based surveys reported that African immigrant population living in France is subjected to a very high risk of being denied care by health-care professionals (27, 32, 33).

Study limitations

Our study has some limitations. Firstly, since the inclusion sites were limited to HIV and STI outpatient consultation service of a healthcare facility in Orléans, this may have introduced a recruitment bias in favor of STI- and HIV-infected women. Therefore, healthy African immigrant women might be under-represented in our study. In addition, the high frequency of HIV-infected women in our study could lead to an overestimation of the burden of HPV infection. Finally, the short sample size of our study population makes these results not fully representative of the first-generation immigrant women originating from sub-Saharan Africa and living in France.

New contribution to the literature

Our study highlights for the first time the high prevalence of cervical HPV infection with original genotypes distribution frequently associated with HIV infection in under-screened immigrant women originating from sub-Saharan Africa and living in France. There is an urgent need to implement adapted screening interventions specific for sub-Saharan African immigrant women who appear to be at very high-risk for cervical cancer. Regular access to HPV molecular screening and Pap smear triage constitutes likely the necessary condition to allow preventing genital cancers in immigrant African women living in France. New strategies such as vaginal self-sampling for molecular HPV test have proven to be as suitable for molecular test as the usual clinician-administered vaginal sampling (23, 34). Furthermore, vaginal self-sampling is a good alternative to reach remote and neglected population with limited access to healthcare facilities in developed countries (35). Such new strategy effective for women in Africa could be adapted for first-generation African women living in France. Indeed, in a randomized trial conducted in France, the self-sampling strategy was more effective and cost-effective than the recall letter commonly used in France, increasing thus the participation in cervical cancer screening (34). Therefore, offering at social welfare centers, as well as in hot spots of residence of immigrant populations of African origin an in-home return-mail kit vaginal self-sampling device, could help to increase the cervical cancer screening coverage in this key and neglected sub-population of French women. In addition, special attention should be furthermore focused on HIV-infected immigrant women, since HIV constitutes an aggravating factor for the progression of HPV-related lesions to invasive cancer (36). Finally, further investigations are necessary to better identify cervical screening barriers specific to the sub-Saharan African immigrant women living in France, especially those of first-generation who are not eligible for vaccination anymore. Addressing the barriers in screening access and attendance for sub-Saharan African women living in France may be pivotal in further decreasing the incidence and mortality associated to cervical cancer in this vulnerable and neglected population.

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3. Distribution anale inhabituelle et unique de géotypes à haut-risque de Papillomavirus humains (HR-HPV) parmi des hommes ayant des rapports sexuels avec des hommes vivant en République Centrafricaine.

Cette partie traite de l'épidémiologie de l'infection à papillomavirus humains (HPV) et les principaux facteurs de risque associés, au sein d'une population d'hommes ayant des rapports sexuels avec des hommes vivant en République Centrafricaine. Ce travail de recherche a fait l'objet d'une publication intitulée: *“Unusual and unique distribution of anal high-risk human papillomavirus (HR-HPV) among men who have sex with men living in the Central African Republic”* parue dans le journal scientifique *“Public Library of sciences (Plos One)”*, le 24 mai 2018.

Avant-propos.

Rationnel et objectif de l'étude. L'infection à Papillomavirus humain à haut risque (HR-HPV) demeure une grande préoccupation pour les hommes africains qui ont des relations sexuelles avec des hommes (HSH), en particulier ceux infectés par le VIH qui constituent le groupe le plus à risque pour l'infection à HR-HPV (Park et al., 2013; Keshinro et al., 2016). Cependant, l'épidémiologie de l'infection à HPV chez les HSH en Afrique sub-Saharienne demeure très mal connue car peu documentée. Néanmoins, les observations faites dans quelques pays Africains au sein de la population HSH démontrent que cette population constitue un groupe de transmission de plusieurs infections sexuellement transmissibles et donc très à risque pour l'infection anale à HR-HPV. D'autre part, ces observations laissent supposer que la distribution des géotypes de HPV circulant au sein de cette population pourrait être relativement différente à celle observée dans la population générale, et dans d'autres continents (Müller et al., 2016; Nowak et al., 2016). Afin de vérifier ces hypothèses, nous avons dressé, dans une étude observationnelle et transversale, une estimation de la prévalence de l'infection anale à HR-HPV et identifié les facteurs de risque associés au sein d'un échantillon de HSH vivant à Bangui, en République Centrafricaine.

Méthodologie. Les HSH soignés au Centre national de référence des infections sexuellement transmissibles et de la thérapie antirétrovirale de Bangui ont été inclus dans l'étude. Le statut sérologique vis-à-vis de l'infection à VIH et les caractéristiques sociodémographiques et comportementales des patients ont été recueillis. La détection de l'infection anale à HPV a été

réalisée à partir d'écouvillons anaux en utilisant le système de PCR en temps réel multiplex Anyplex II HPV28 genotyping test (Seegene, Seoul, Corée du Sud). Les HSH inclus ont également subi un dépistage moléculaire de l'infection anale à HSV-2 par PCR en temps réel. Enfin, une analyse de régression logistique a été utilisée pour déterminer les facteurs de risque (sociodémographiques, comportementaux et cliniques) associés aux résultats du dépistage anal de l'infection à HPV.

Résultats. Quarante-deux (42) HSH [âge moyen : 23,2 (14-39) ans], dont 69,1% étaient infectés par le VIH et 30,9% séronégatifs pour l'infection à VIH, ont été inclus de manière prospective dans l'étude. La prévalence générale de l'infection anale à HPV était de 69,1%, dont 82,7% représentaient des infections causées par des HR-HPV, 52,0% des infections anales causées par plusieurs génotypes différents. Les génotypes les plus répandus étaient HPV-35, HPV-58, HPV-59 et HPV-31. Les génotypes HPV-16 et HPV-18 étaient présents dans une minorité d'échantillons. Les infections causées par plusieurs génotypes de HR-HPV à la fois étaient plus fréquentes chez les HSH séropositifs pour le VIH (41,4%) avec en moyenne 2,7 génotypes par échantillon, comparé aux HSH VIH-négatifs (7,7%) avec en moyenne 1,5 génotypes par échantillon. Les génotypes de HPV ciblés par le vaccin prophylactique Gardasil-9® ont été détectés dans 68,9% des échantillons et le HPV-58 était le génotype le plus fréquemment détecté. Les HSH infectés par du HPV-16 et HPV-18 étaient tous infectés par le VIH. D'autres part, il n'y avait que peu de prélèvements anaux (11,9%) qui étaient positifs pour l'infection à HSV-2. L'infection anale à HSV-2 ne constituait pas, dans cet échantillon de HSH, un facteur de risque pour l'infection anale à HR-HPV. Par contre, nous avons pu démontrer que les rapports sexuels anaux réceptifs non protégés représentaient le principal facteur de risque pour l'infection anale à HPV (Odd ratios = 14,1) contrairement aux rapports sexuels anaux insertifs, qui eux étaient significativement associé à une faible prévalence de l'infection anale à HPV (Odd ratios ajusté = 0,002).

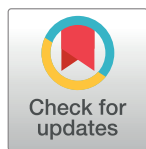
Conclusion. Les HSH vivant à Bangui, en République Centrafricaine sont à risque pour l'infection à la fois par le VIH et les HR-HPV. La distribution inhabituelle avec le génotype HPV-35 représentant le génotype prédominant suggère des spécificités géographiques possibles dans l'épidémiologie moléculaire de l'infection HR-HPV en Afrique subsaharienne et particulièrement chez les HSH Africains. La priorité doit être donnée aux stratégies de prévention de l'infection à HPV et des cancers associés adaptées aux HSH en Afrique. Des interventions innovantes devraient élaborées pour la population de HSH vivant à Bangui en République Centrafricaine.

RESEARCH ARTICLE

Unusual and unique distribution of anal high-risk human papillomavirus (HR-HPV) among men who have sex with men living in the Central African Republic

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Abstract

Background

High-risk (HR) human papillomavirus (HPV) infection remains a great concern in relation to African men who have sex with men (MSM), especially those infected with HIV. The prevalence of HR-HPV and associated risk factors was estimated in a cross-sectional observational study covering MSM living in Bangui, Central African Republic.

Methods

MSM receiving care at the *Centre National de Référence des Infections Sexuellement Transmissibles et de la Thérapie Antirétrovirale*, Bangui, were included. HIV serostatus and socio-demographic and behavioral characteristics were collected. HPV DNA was detected and genotyped on anal swabs using Anyplex™ II HPV28 test (Seegene, South Korea), and HSV DNA by in-house real-time PCR. Logistic regression analyses were used to determine risk factors associated with HPV outcomes.

Results

42 MSM (mean age, 23.2 years; range, 14–39) including 69.1% HIV-1-positive and 30.9% HIV-negative were prospectively enrolled. The prevalence of anal HPV was 69.1%,

including 82.7% of HR-HPV which were multiple in 52.0%. The most prevalent genotypes were HPV-35, HPV-58, HPV-59 and HPV-31. While, HPV-16 and HPV-18 were present in a minority of samples. Multiple HR-HPV infection was more frequent in HIV-positive MSM (41.4%) with 2.7 genotypes per anal samples than in HIV-negative (7.7%) with 1.5 genotypes per anal samples. HPV types included in the prophylactic Gardasil-9[®] vaccine were detected in 68.9% of specimens and HPV-58 was the most frequently detected. MSM infected by HPV-16 and HPV-18 were all infected by HIV-1. Few anal swabs (11.9%) contained HSV-2 DNA without relationship with HPV detection. Condomless receptive anal intercourse was the main risk factor to being infected with any type of HPV and condomless insertive anal intercourse was significantly less associated with HPV contamination than receptive anal intercourse (Odd ratio = 0.02).

Conclusion

MSM in Bangui are at-risk of HIV and HR-HPV anal infections. The unusual distribution of HPV-35 as predominant HPV suggests possible geographic specificities in the molecular epidemiology of HR-HPV in sub-Saharan Africa. Scaling up prevention strategies against HPV infection and related cancers adapted for MSM in Africa should be prioritized. Innovative interventions should be conceived for the MSM population living in Bangui.

Introduction

Men who have sex with men (MSM) in sub-Saharan Africa constitute a core group for several sexual transmitted infections (STI), including human immunodeficiency virus (HIV) [1–4], human papillomavirus (HPV) and herpes simplex virus type 2 (HSV-2) infections [5–8]. HPV infection is the most common viral STI in the world and high-risk oncogenic (HR)-HPV genotypes are responsible for 7.7% of all cancers in developing countries [9,10]. Therefore, anal HR-HPV infection is a steady increasing health problem for MSM because it causes anal cancer. Indeed, MSM are about 20 times more likely susceptible than heterosexual men to develop HPV-related anal cancer and HIV-infected MSM are at an even greater risk [11,12]. Indeed, HIV infection is considered as an independent risk factor strongly associated with increased risk for acquiring HR-HPV anal infection [13–15]. *In vitro* interactions between HSV-2 and HPV suggest that HSV-2 infection could constitute a cofactor of anal carriage for HPV infection [16], especially in the epidemiological context of sub-Saharan Africa, where HSV-2 infection is highly prevalent [17,18], and constitutes the first cause of genital ulcer [17–19].

The burden of HPV infection in MSM living in sub-Saharan Africa has nonetheless been poorly documented [8]. However, recent reports from South Africa [6] and Nigeria [7] emphasize very high prevalences of anal HR-HPV infection, ranging from 57.6 to 70.1%, in MSM living in sub-Saharan Africa, especially in those co-infected with HIV [6,7]. The reported prevalences of anal HR-HPV DNA among MSM living in sub-Saharan Africa appear higher than those usually recorded in studies conducted in developed countries, which range from 20.9% to 65% [20–23]. Anal HR-HPV in African MSM was strongly associated with high-risk sexual behaviors such as having sex with men only, engaging in group sex and practicing condomless receptive anal intercourse [6,7]. Interestingly, a wide diversity of predominant HPV genotypes was observed in South African and Nigerian studies, suggesting the possibility of unique spatial distributions of HPV diversity by regions within sub-Saharan Africa [6,7]. Thus, Müller

et al. described in South Africa a distribution quite similar to that commonly observed throughout the world with HPV-16 as predominant genotype [6]. In contrast, Nowak et al. depicted in Nigeria an atypical distribution profile with the non-vaccine HR-HPV-35 as the predominant genotype circulating in MSM [7]. Although limited, these observations highlight that MSM in sub-Saharan Africa constitute a high-risk core group for HR-HPV infection and that the distribution of the main HPV genotypes involved in anal cancers in African MSM could be relatively different from that generally observed. Finally, in order to implement effective HPV vaccine-based prevention adapted to every sub-Saharan African region, it is important to establish the molecular distribution of predominant HR-HPV genotypes circulating in African MSM.

Little data is available on MSM living in the Central African Republic (CAR). One recent preliminary serosurvey conducted on MSM in Bangui highlighted that MSM are an identifiable core group accumulating high-risk sexual behaviors for STI, and high prevalence of HIV (25%), hepatitis B (17%) and syphilis (4%) [24]. Herein, we designed a cross-sectional study to assess the prevalence and type distribution of anal HPV infection and associated risk factors, including sexual behavior and HSV-2 infection, in a population of HIV-infected and HIV-uninfected MSM living in Bangui, the capital city of the CAR.

Material and methods

Study population, medical interventions and data collection

The Centre National de Référence des Infections Sexuellement Transmissibles et de la Thérapie Antirétrovirale (CNRIST/TAR) of Bangui includes both care for general population and specific care towards the MSM population of Bangui. MSM regularly attend the STI clinic for HIV and STI screening and care, to receive specific treatment, HIV counseling and for those positive, for HIV global support. For purposes of the study, a specific strategy involving peer educators was adopted in order to confirm the accuracy of homosexuality of the included MSM attending the CNRIST/TAR. Thus, inclusion criteria were to be in majority age (age ≥ 18 years), to be approved as having sex with men by his peers, to get possible follow up for at least 3 months, and to have a fully informed medical and socio-demographic record. At inclusion, a standardized interview was conducted to collect socio-demographic characteristics and behavioral data, including age, ethnic group, number of sexual male partners in the last 6 months, sexual orientation, frequency of condom use, sexual practices, HIV status and antiretroviral treatment (ART) for those already aware of their positive HIV status and finally to advise participants about HIV and associated STIs.

After the interviews, MSM undertook medical appointments including clinical examinations and biological investigations for the diagnosis of the most common STIs including HIV (for those who did not know their HIV-serological status), syphilis and hepatitis B. Biological results were returned 72 hours after and those positive for STIs received adapted treatment. HIV-positive MSM were enrolled in the HIV cohort followed in the CNRIST/TAR. The medical intervention package consisted of HIV/STI counseling, condom distribution, clinical examination, biological monitoring, and medical care for patients infected with STIs and HIV. A Medical professional carried out physical and clinical examinations to check patients for symptoms of potential diseases. For HIV/STI counseling and condom distribution, a 7 to 10-minute interactive conversation on HIV, STIs, their modes of transmission and effective prevention mean with a special emphasis on condom use as an easy and effective prevention tool, was carried out by health care assistants. At the end, the health care assistants distributed as many condoms as required to the participants.

Samples and processing

Plasma or serum samples from blood collected by venipuncture in each MSM were used for serological testing of HIV infection, as recommended by national guidelines [25]. HIV-positive status was determined by the national serological testing algorithm using Genscreen ULTRA Combo HIV Ag/Ab test (Bio-Rad Laboratories, Washington, USA), an enzyme immunoassay kit for the simultaneous detection of HIV p24 antigen and antibodies to HIV-1 (group M and O) and HIV-2 in serum or plasma. The positive tests were confirmed by an enzyme-linked immunosorbent assay [ELISA] (Vironostika® HIV Uni-Form II Ag/Ab, bio-Mérieux Marcy l'Etoile, France). Specimens for molecular testing were obtained by inserting a moistened polyester swab into the anal canal, rotating 5 times and then removing. The swab was immediately placed into a sampler tube and frozen at -80°C before the DNA extraction procedure.

HPV detection and genotyping

DNA was extracted from the anal swab specimen using the DNeasyBlood and Tissue kit, as recommended by the manufacturer (Qiagen, Hilden, Germany). The detection of HPV DNA and the distribution of genotypes were done using Anyplex™ II HPV28 detection test (Seegene, Seoul, South Korea). Anyplex™ II HPV28 detection test was performed as recommended by the manufacturer with 5 µl of DNA in each of the two reaction mixtures (20 µl) with the primers A and B [26]. According to the International Agency for Research on Cancer (IARC) nomenclature [27], Anyplex™ II HPV28 detection test distinguishes 28 HPV genotypes, including 13 high-risk types (HR-HPV -16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68), 9 low-risk (LR) types (LR-HPV -6, -11, -40, -42, -43, -44, -53, -54 and -70) and then, 6 genotypes reported as possibly carcinogenic (HPV-26, -61, -66, -69, -73 and -82). The process is carried out in 2 reactions by taking advantage of the 5 dyes that can be resolved on the CFX96™ real-time PCR instrument (Bio-Rad, Marnes-la-Coquette, France) [26]. Anyplex™ II HPV28 has been evaluated for several years and is as suitable for HPV genotyping as other molecular assays commonly used for genotyping [26, 28–32]. Data recording and interpretation were automated with Seegene viewer software (Seegene, Seoul, South Korea), according to the manufacturer's instructions. A swab sample was considered positive for any HPV if containing any of the 28 types included in the Anyplex™ II HPV28 detection test; positive for multiple HPV when containing at least 2 types of the 28 HPV types included in genotypic test; HR-HPV positive and multiple HR-HPV positive when containing respectively at least 1 HR-HPV type and at least 2 HR types of the 19 HR types of the Anyplex™ II HPV28 detection test, irrespective of the presence of LR-HPV.

HSV detection in the anal tract

HSV DNA was detected from 1 µg of DNA extracted from the anal swab specimen by in-house real-time PCR using LightCycler® 480 Real-Time PCR (Roche molecular diagnostics, California, USA), as previously described [33,34]. HSV genotypes (HSV-2 versus HSV type 1) were assessed by the final melting curve, [33,34].

Statistical analyses

Statistical analyses were conducted using IBM® SPSS® Statistics 20 software (IBM, SPSS Inc, Armonk, New York, USA). P-values were calculated using Pearson's χ^2 test or Fisher's exact test for categorical variables and the non-parametric Mann-Whitney *U*-test for quantitative variables. Logistic regression models were assessed to evaluate the association of each

independent variable [i.e., age at enrollment, HIV-1 infection, sex of sexual partner (MSM-exclusively or MSMW), the number of sexual male partners in the last 6 months, sexual practices in the last 6 months (condomless receptive anal sex; condomless insertive anal sex; regular receptive oral sex) and the HSV-2 DNA detection in anal swab] with the HPV type-specific anal infections (i.e., anal infection by any type of HPV, multiple types of HPV, HR-HPV and multiple HR-HPV). All variables statistically significant ($P < 0.05$) in univariate analyses were entered into multivariate logistic regression models. Crude Odds ratio (cOR) and adjusted Odds ratio (aOR) were calculated, as appropriate along with 95% confidence intervals (CI). For variable giving infinite OR, the Odds ratios and their confidence intervals were recalculated, using the statistical software package R (available at <https://www.r-project.org/>) and the hypothesis test inversion method, as previously described [35]. The final multivariate model for any HPV outcome included condomless receptive and insertive anal sex and regular receptive oral sex. For multiple HPV outcome, the final multivariate model included condomless receptive and insertive anal sex and regular receptive oral sex. For HR-HPV outcomes, the final multivariate model included condomless insertive anal sex and regular receptive oral sex. Finally, for the multiple HR-HPV outcomes variable, the final multivariate model included HIV infection and condomless insertive anal sex.

Ethics statement

The study was formally approved by the Scientific Committee Faculty of Health Sciences of Bangui ("Comité Scientifique Chargé de la Validation des Protocoles d'Etudes et des Résultats"/"CSCVPER") (agreement UB/FACSS/CSCVPER), which constitutes the National Ethical Committee. All MSM participants were of majority age and gave their informed oral consent to participate in the study. For each MSM, the record of the consent was documented in each questionnaire. This consent procedure was formally approved by the National Ethical Committee.

Results

Characteristics of study population

Forty-two participants were included and their socio-demographic, sexual behavior, clinic-biological characteristics are shown in the [Table 1](#). Among them, 29 (69.1%) were infected by HIV-1 whereas 13 (30.9%) were HIV-negative.

Overall, the study population was exclusively constituted by black native people and comprised mainly young men (mean age: 23.2 years; range, 18–39). All were living in 4 (out of 10) districts of the capital city Bangui which were neighboring. The majority of participants (34/42; 80.9%) reported having sex in the last 6 months with both men and women (MSMW), whereas a minority (8/42; 19.1%) reported having sex in the last 6 months exclusively with men (MSM-exclusively). The prevalence of HIV-1 infection was higher in MSM-exclusively (8/8; 100%; 95%CI: 100–100%) than in MSMW (21/34; 61.7%; 95%CI: 45.4–78.1%) ($P = 0.04$). At inclusion, only 10 of 29 (34.5%) HIV-infected MSM, including 4 MSM-exclusively and 6 MSMW, were taking antiretroviral treatment (ART) according to the 2015-World Health Organization (WHO) consolidated guidelines [36]. Among them, only 6, including 4 MSM-exclusively and 2 MSMW, showed a baseline CD4 cell count above 500 cells/ μ l. Most (40/42; 95.3%) of the MSM reported having sexual intercourse with at least 1 to 5 partners (median: 4; range, 1–5) in the last 6 months and most of them (30/42; 71.4%) had condomless receptive anal sex during the past 6 months. The group of MSM-exclusively reported having 7-time more receptive anal sex than insertive anal sex [receptive anal sex: 7/8 (87.5%), 95%CI: 64.6–100.0%; insertive anal sex: 1/8 (12.5%), 95%CI: 0.0–35.4%]. Furthermore, MSM-exclusively reported more likely to have receptive oral sex than MSMW [MSM-exclusively: 8/8 (100.0%),

Table 1. Baseline characteristics according to sexual behavior and HIV serostatus among the 42 study men who have sex with men (MSM) living in Bangui, Central African Republic.

	MSM	MSM-exclusively	MSMW	P [*]	HIV+	HIV-	P [*]
Number of patients	42	8	34		29	13	
Age							
All age [mean (SD), years]	23.2 (5.6)	20.5 (0.7)	23.2 (4.9)	NS	19.5 (4.9)	21.5 (5.6)	NS
18–19	11 (26.2)	2 (25) [0.0–55.1]	9 (26.5) [11.6–41.3]	NS	5 (17.3) [3.5–30.9]	6 (46.2) [19.1–73.3]	NS
20–29	23 (54.7)	5 (62.5) [28.9–96.1]	18 (64.7) [36.2–69.7]		18 (62.1) [44.4–79.7]	5 (38.5) [12.1–64.9]	
≥30	8 (19.1)	1 (12.5) [0.0–35.4]	7 (8.8) [7.0–34.2]		6 (20.7) [5.9–35.4]	2 (15.4) [0.0–35.0]	
HIV-1 infection [n (%)]	29 (69)	8 (100) [100–100]	21 (61.7) [45.4–78.1]	0.04	NA	NA	NA
ART at inclusion [n (%)]	10 (23.8)	4 (50) [15.4–84.6]	6 (17.4) [4.8–30.5]	0.07	10 (34.5)	NA	NA
CD4 T cell counts [n (%),cells/μl]							
> 500	13 (30.9)	4 (50.0) [15.4–84.6]	9 (42.8) [21.7–64.1]	NS	13 (44.8)	NA	NA
351–500	13 (30.9)	3 (37.5) [3.9–71.1]	10 (47.6) [26.3–68.9]		13 (44.8)	NA	
≤ 350	3 (7.2)	1 (12.5) [0.0–35.4]	2 (9.5) [0.0–22.1]		3 (10.4)	NA	
Sex of sexual partners over the last 6 months [n (%)]							
MSM-exclusively	8 (19.1)	NA	NA	NA	8 (27.6) [11.3–43.8]	0 (0) [0.0–0.0]	0.04
MSMW	34 (80.9)	NA	NA		21 (72.4) [56.2–88.7]	13 (100) [100–100]	
Numbers of sexual male partners in last 6 months [n (%)]							
1–5	40 (95.3)	8 (100) [100–100]	32 (94.1) [86.2–100]	NS	28 (96.5) [89.9–100]	12 (92.3) [77.8–100]	NS
> 5	2 (4.7)	0 (0) [0.0–0.0]	2 (5.9) [0.0–13.8]		1 (3.5) [0.0–10.1]	1 (7.7) [0.0–22.2]	
Sexual practices in last 6 months [n (%)]							
Condomless receptive anal sex	30 (71.4)	7 (87.5) [64.6–100]	23 (67.6) [51.9–83.4]	NS	22 (75.8) [60.3–91.4]	8 (61.5) [35.1–87.9]	NS
Condomless insertive anal sex	15 (35.7)	1 (12.5) [[0.0–35.4]	14 (41.2) [24.6–57.7]	NS	10 (34.5) [17.18–51.78]	5 (38.5) [12.1–64.9]	NS
Regular receptive oral sex	28 (66.6)	8 (100) [100–100]	20 (58.8) [42.3–75.4]	0.03	25 (86.2) [73.6–98.7]	3 (23.1) [0.2–45.9]	0.0001
HSV-2 DNA in swab [n (%)]	5 (11.9)	1 (12.5) [0.0–35.4]	4 (11.7) [0.9–22.6]	NS	5 (17.3) [3.5–30.9]	0 (0) [0.0–0.0]	NS
HPV DNA detection and types [n (%)]							
HPV DNA in swab	29 (69.1)	7 (87.5) [64.6–100.0]	22 (64.7) [48.6–80.7]	NS	21 (72.4) [56.15–88.68]	8 (61.5) [35.1–87.9]	NS
Multiple types of any HPV	25 (59.5)	7 (87.5) [64.6–100.0]	18 (52.9) [36.2–69.7]	NS	19 (65.5) [48.22–82.82]	6 (46.2) [19.1–73.3]	NS
LR-HPV	20 (47.6)	5 (62.5) [28.9–96.1]	15 (44.1) [27.4–60.8]	NS	14 (48.2) [30.1–66.4]	6 (46.2) [19.1–73.3]	NS
HR-HPV	24 (57.1)	5 (62.5) [28.9–96.1]	19 (55.8) [39.2–72.6]	NS	18 (62.1) [44.4–79.7]	6 (46.2) [19.1–73.3]	NS
Multiple types of HR-HPV	13 (30.9)	3 (37.5) [3.9–71.1]	10 (29.4) [14.1–44.7]	NS	12 (41.4) [23.5–59.3]	1 (7.7) [0.0–22.2]	0.007
HPV-16	4 (9.5)	2 (25.0) [0.0–55.1]	2 (5.8) [0.0–13.8]	NS	4 (13.8) [1.2–26.3]	0 (0) [0.0–0.0]	NS
HPV-18	3 (7.2)	1 (12.5) [0.0–35.4]	2 (5.8) [0.0–13.8]	NS	3 (10.3) [0.0–21.4]	0 (0) [0.0–0.0]	NS
HPV-16 and HPV-18	1 (2.3)	1 (12.5) [0.0–35.4]	0 (0) [0.0–0.0]	NS	1 (3.4) [0.0–10.1]	0 (0) [0.0–0.0]	NS
Any 4-valent vaccine types**	11 (26.2)	4 (50.0) [15.4–84.6]	7 (20.6) [7.0–34.2]	NS	9 (31.1) [14.2–47.8]	0 (0) [0.0–0.0]	0.04
Multiple 4-valent vaccine types	1 (2.3)	1 (12.5) [0.0–35.4]	0 (0) [0.0–0.0]	NS	1 (3.5) [0.0–10.1]	0 (0) [0.0–0.0]	NS
Any 9-valent vaccine types***	20 (47.6)	4 (50.0) [15.4–84.6]	16 (47.1) [30.3–63.8]	NS	15 (51.7) [33.6–69.9]	5 (38.5) [12.2–64.9]	NS
Multiple 9-valent vaccine types	9 (21.5)	2 (25.0) [0.0–55.1]	7 (20.6) [7.0–34.2]	NS	7 (24.2) [8.5–39.7]	2 (15.4) [0.0–35.0]	NS

* P-value calculated using Pearson’s χ^2 test or Fisher’s exact test for categorical variables and the non-parametric Mann-Whitney U-test for non-categorical variables.

** The 4-valent Gardasil-4® vaccine (Merck & Co. Inc., New Jersey, USA) is effective against HPV genotypes 6, 11, 16 and 18;

*** The 9-valent Gardasil-9® vaccine is effective against HPV genotypes 6, 11, 16, 18, 31, 33, 45, 52 and 58.

ART: Antiretroviral treatment; HIV-1: Human immunodeficiency virus-1; HSV-2: Herpes simplex virus-2; HR-HPV: high-risk human papillomavirus; LR-HPV: low-risk human papillomavirus; MSM: men who have sex with men; MSM-exclusively: men who have sex only with men; MSMW: men who have sex with both men and women; NA: Not applicable; NS: Not significant.

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95%CI: 100.0–100.0%; MSMW: 20/34 (58.8%), 95%CI: 42.28–75.37%; $P < 0.03$]. Lastly, HIV-infected MSM reported practicing more regularly receptive oral sex than HIV-negative MSM [25/29 (86.2%); 95%CI: 73.6–98.7% *versus* 3/13 (23.1%); 95%CI: 0.2–45.9%; $P < 0.001$].

Finally, the study MSM were generally free of clinical STI symptoms at admission. Thus, only 5 MSM showed herpetic genital recurrences (n = 2), syphilis genital ulcer (n = 1), anal warts (n = 1) and anal infection due to *Neisseria gonorrhoea* (n = 1). The seroprevalences of syphilis and hepatitis B (HBs antigen) at inclusion were 12% and 14%, respectively.

HSV DNA detection

Only 5 (11.9%) of the study MSM were positive for HSV-2 in anal samples (1 MSM-exclusively and 4 MSMW); all of them were coinfecting with HIV-1 and 4 of them showed multiple anal HR-HPV infections.

HPV prevalence and genotype distribution

As shown in Table 1, the overall HPV prevalence in study population was 69.1% (29/42) with 82.7% (24/29) of HR-HPV DNA-positive samples. Most (25/42; 59.5%) anal swabs contained multiple HPV genotypes and 52.0% (13/25) of them contained an average of 2.7 HR-HPV (range, 1 to 4) per anal swab sample. About 24 out of 29 (82.7%; 95%CI: 69.01–96.51%) HPV-positive anal specimens showed at least 1 HR-HPV and only 37.9% (11/29) showed single HR-HPV infections. The distribution of HPV genotypes in HPV DNA-positive anal samples is depicted in Fig 1.

HR-HPV-35 was the predominant genotype (8/29; 27.6%), followed by LR-HPV types 42 and 53 with a prevalence of 24.1% (7/29), HR-HPV types 58 and 59 with 20.7% (6/29) and HR-HPV types 31 and 61 with 17.2% (5/29). HPV-16 and HPV-18 were found in a minority of HPV-positive swabs [4/29 (13.8%) and 3/29 (10.3%), respectively] (Fig 1).

Among the 21 HIV-1-infected MSM with anal HPV infection, 19 (90.5%) had multiple HPV and 20 (95.3%) were co-infected with HR-HPV (Fig 2 and Table 1). Multiple anal HR-HPV types were detected more frequently in HIV-1-infected than HIV-negative MSM (41.4% versus 7.7%; P<0.01). The mean number of HR-HPV genotypes more frequently detected in HR-HPV-positive anal swabs was 2.7 (range 1–4) in HIV-positive MSM and 1.5 (range 1–4) in HIV-negative MSM (P = 0.07). MSM positive for HPV-16, HPV-18 and HPV-

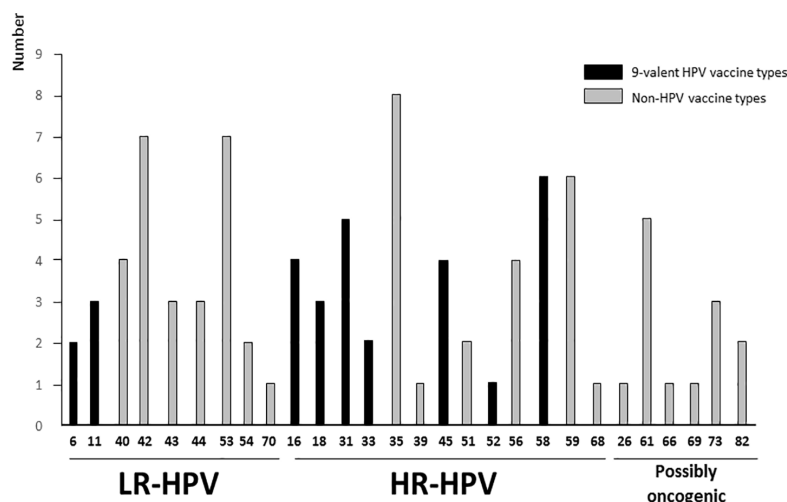


Fig 1. Distribution of anal HPV types included (or not included) in the 9-valent Gardasil-9® vaccine. Number of low-risk (LR) and high-risk (HR) HPV genotypes in 29 anal swabs positive for HPV DNA by molecular biology according to their possible prevention by 9-valent HPV vaccine among study men who have sex with men (n = 42) living in the Central African Republic. Nota bene. The 9-valent Gardasil-9® vaccine (Merck & Co. Inc., New Jersey, USA) is effective against HPV genotypes 6, 11, 16, 18, 31, 33, 45, 52 and 58.

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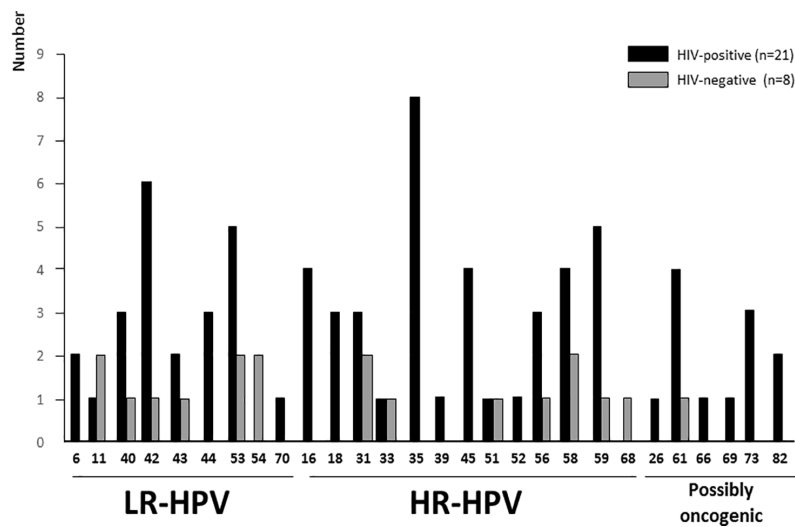


Fig 2. Distribution of anal HPV types according to the HIV-serostatus. Number of low-risk (LR) and high-risk (HR) HPV genotypes in 29 anal swab positive for HPV DNA by molecular biology according to HIV-serostatus among study men who have sex with men (n = 42) living in the Central African Republic.

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35 were all HIV-1-infected (Fig 2) and only one sample was simultaneously infected with both HPV-16 and HPV-18.

Possible efficiencies of anal HPV prevention by 4- and 9- valent Gardasil® vaccines were further assessed. 37.9% (11/29) of the HPV-positive anal swabs were infected with at least 1 of the 4 genotypes covered by the Gardasil-4 vaccine. Regarding the Gardasil-9 vaccine, 68.9% (20/29) of HPV-positive anal samples contained at least 1 HPV type included in the 9-valent HPV vaccine and 45% (9/20) of them contained multiple HPV genotypes (Fig 2 and Table 1). The predominant HR-HPV genotype included in the 9-valent HPV vaccine detected in study anal swabs was the type 58 with a prevalence of 20.7% (20/29) (Fig 2).

Risk factors associated with HPV infection. The associations between anal HPV infection, including anal infection by any type of HPV, multiple types of HPV, and HR-HPV type and multiple types HR-HPV, with their potential risk factors were assessed by logistic regression analysis, as shown in Table 2.

No significant differences could be observed between MSM-exclusively and MSMW regarding to the prevalence of HPV DNA detection in the anal samples [7/8 (87.5%) versus 22/34 (64.7%), respectively; $P > 0.05$]. However, the MSM-exclusively group had the highest rates of anal detection of HPV infections, including LR-HPV and HR-HPV with high prevalence of HPV-16 and HPV-18, multiple HPV as well as HPV types included in the 9-valent HPV prophylactic vaccine.

In the univariate analysis, anal infections by any type of HPV and multiple type of HPV were significantly associated with the practice of condomless receptive anal sex (cOR: 14.1, 95%CI: 2.9–68.4%; $P = 0.001$; cOR: 5.9, 95%CI: 1.4–24.7%; $P = 0.014$, respectively). Likewise, anal infections by any type of HPV, multiple type of HPV and anal infections with HR-HPV were significantly associated with the practice of receptive oral sex (cOR: 11.0, 95%CI: 2.3–49.5%; $P = 0.002$; cOR: 7.5, 95%CI: 1.8–31.7%; $P < 0.05$ and cOR: 16.9, 95%CI: 3.4–83.7%; $P < 0.001$, respectively). The anal carriage of multiple anal HR-HPV infection was significantly associated with being infected with HIV (cOR: 15.0, 95%CI: 1.4–161.1%; $P = 0.003$). Insertive anal sex was significantly associated with a slightly decreased risk of being infected with any HPV type, HR-HPV type and multiple HR-HPV type in the anal canal (cOR: 0.01, 95%CI: 0.002–0.130%; $P < 0.01$; cOR: 0.03, 95%CI: 0.0–0.2%; $P < 0.001$ and cOR: 0.01, 95%CI: 0.02–0.65%; $P = 0.008$, respectively).

Table 2. Univariate and multivariate logistic regression analyses for HPV-associated risk factors 42 study men who have sex with men (MSM) living in Bangui, Central African Republic.

Risk factors	Any HPV					Multiple HPV					HR-HPV					Multiple HR-HPV					
	29					25					24					13					
	n (%)	cOR (95%CI)	P*	aOR (95%CI)	P*	n (%)	cOR (95%CI)	P*	aOR (95%CI)	P*	n (%)	cOR (95%CI)	P*	aOR (95%CI)	P*	n (%)	cOR (95%CI)	P*	aOR (95%CI)	P*	
Age (years)																					
18–19	8 (27.6)	0.7 (0.16–3.5)	0.9	NA**	NA	8 (32)	2.2 (0.5–9.9)	0.3	NA	NA	5 (19.2)	0.4 (0.1–1.6)	0.4	NA	NA	2 (12.5)	0.9 (0.2–3.8)	0.8	NA	NA	
20–29	14 (48.2)	1.13 (0.1–15.5)		NA	NA	12 (48)	0.9 (0.3–3.5)		NA	NA	13 (50.0)	1.8 (0.5–6.4)		NA	NA	8 (50.0)	1.4 (0.4–5.1)		NA	NA	
≥ 30	7 (24.1)	1.7 (0.1–20.3)		NA	NA	5 (20)	0.2 (0.0–2.1)		NA	NA	6 (23.5)	1.9 (0.2–20.6)		NA	NA	3 (18.7)	0.5 (0.05–5.4)		NA	NA	
HIV-1 infection																					
	21 (74.4)	2.25 (0.6–8.7)	0.2	NA	NA	19 (76)	3.16 (0.3–27.6)	0.3	NA	NA	20 (76.9)	3.3 (0.9–12.7)	0.07	NA	NA	15 (93.7)	15.0 (1.4–161.1)	0.003	5.2 (0.4–70.9)	0.2	
Sex of sexual partners																					
MSM-exclusively																					
	7 (24.2)	3.8 (0.4–34.8)	0.2	NA	NA	7 (28)	6.2 (0.7–56.2)	0.08	NA	NA	6 (23.1)	0.6 (0.04–7.8)	0.6	NA	NA	4 (25)	1.33 (0.2–9.1)	0.7	NA	NA	
MSMW																					
	22 (75.8)	0.3 (0.03–2.4)	0.2	NA	NA	18 (72)	0.2 (0.0–1.5)	0.08	NA	NA	20 (76.9)	1.6 (0.12–21.7)	0.6	NA	NA	12 (75)	0.75 (0.11–5.1)	0.7	NA	NA	
Numbers of sexual male partners in last 6 months																					
1–5	27 (93.1)	0.0 (0.0–100.0)	0.9	NA	NA	23 (92)	0.0 (0.0–100.0)	0.9	NA	NA	24 (92.3)	0.0 (0.0–100.0)	0.4	NA	NA	14 (87.5)	0.0 (0.0–100.0)	0.9	NA	NA	
> 5	2 (6.8)	0.0 (0.0–100.0)		NA	NA	2 (8)	0.0 (0.0–100.0)		NA	NA	2 (7.7)	0.0 (0.0–100.0)		NA	NA	2 (12.5)	0.0 (0.0–100.0)		NA	NA	
Sexual practices in last 6 months																					
Condomless insertive anal sex																					
	4 (13.8)	0.01 (0.002–0.13)	< 0.001	0.02 (0.001–0.18)	< 0.01	3 (12)	0.4 (0.03–5.3)	< 0.05	0.05 (0.01–0.3)	0.01	3 (11.5)	0.03 (0.0–0.2)	<0.001	0.022 (0.0–0.24)	0.002	2 (12.5)	0.1 (0.02–0.65)	0.008	0.2 (0.02–1.1)	0.05	
Condomless receptive anal sex																					
	29 (100)	14.1 (2.9–68.4)	0.001	4.9 (0.5–47.1)	0.16	25 (100)	5.9 (1.4–24.7)	0.014	1.4 (0.2–11.3)	0.67	24 (100)	5.0 (1.2–20.6)	0.24	NA	NA	13 (100)	1.7 (0.4–7.8)	0.36	NA	NA	
Regular receptive oral sex																					
	24 (82.7)	11.0 (2.35–49.5)	0.002	10.7 (0.3–118.3)	0.05	21 (84)	7.5 (1.8–31.7)	<0.05	4.8 (0.8–28.2)	0.09	22 (84.6)	16.9 (3.4–83.7)	<0.001	22.3 (2.0–244.1)	0.01	16 (100)	NA	NA	NA	NA	
HSV-2 DNA in swab																					
	4 (13.8)	1.9 (0.2–19.1)	0.57	NA	NA	4 (16)	3.0 (0.3–30.0)	0.9	NA	NA	4 (15.4)	2.7 (0.3–26.9)	0.4	NA	NA	4 (25)	8.3 (0.8–82.9)	0.06	NA	NA	

* P-value calculated using Pearson's χ^2 test or Fisher's exact test for categorical variables and the non-parametric Mann-Whitney U-test for non-categorical variables
 ** NA: Not attributable for variables giving crude Odds ratio not significant in univariate analysis (P > 0.05).

ART: Antiretroviral treatment; aOR: adjusted Odds ratio; cOR: crude Odds ratio; HIV-1: Human immunodeficiency virus-1; HSV-2: Herpes simplex virus-2; HR-HPV: high-risk human papillomavirus; LR-HPV: low-risk human papillomavirus; MSM: men who have sex with men; MSM-exclusively: men who have sex only with men; MSMW: men who have sex with both men and women; n: Number (size of study group); NA: Not attributable; CI: Confidence Interval.

In the multivariate analysis, condomless insertive anal sex and having regular receptive oral sex were the only factors associated with an HPV outcome after adjusting other significant variables in the univariate analysis. Thus, having regular receptive oral sex was a substantial risk factor for being infected with the HR-HPV in the anal canal (aOR: 22.3, 95%CI: 2.0–244.1%; $P = 0.01$). Likewise, the weak protective effect of practicing insertive anal sex against the acquisition of any HPV type, HR-HPV type and multiple HR-HPV type in the anal canal observed in univariate analysis (cOR: 0.01, 95%CI: 0.002–0.13%; $P < 0.01$; cOR: 0.03, 95%CI: 0.0–0.2%; $P < 0.001$ and cOR: 0.1, 95%CI: 0.02–0.65%; $P = 0.008$, respectively) was maintained in multivariate analysis (aOR: 0.02, 95%CI: 0.001–0.18%; $P < 0.01$; aOR: 0.022, 95%CI: 0.0–0.24%; $P = 0.002$ and aOR: 0.20, 95%CI: 0.02–1.1%; $P = 0.05$, respectively). In addition, the effect of insertive anal sex against the acquisition of multiple type of HPV in the anal canal, which was not significant in univariate analysis (cOR: 0.4, 95%CI: 0.03–5.3%; $P < 0.05$) was upgraded as a weak protective effect in the multivariate analysis (aOR: 0.05, 95%CI: 0.01–0.3%; $P = 0.01$). In the other hand the strong risk effect of being infected with any HPV and multiple HPV observed in univariate analysis for MSM practicing condomless receptive anal intercourse was downgraded in multivariate analysis until being not significant anymore (aOR: 4.9, 95%CI: 0.5–47.1%; $P = 0.16$ and aOR: 1.4, 95%CI: 0.2–11.3%; $P = 0.669$; respectively). Finally, the other explicative variables such as age group, sexual orientation (MSM-exclusively or MSMW), number of sexual male partners and anal HSV-2 infection were not significantly associated with each of the four variables characterizing anal HPV infections that were taken into account in the analysis.

Discussion

In the present series, the study of MSM living in the Central African Republic and overseen at the *Centre National de Référence des Infections Sexuellement Transmissibles et de la Thérapie Antirétrovirale* of Bangui showed remarkable findings. Firstly, the prevalence of HIV-1 infection in the MSM was notably high (#70%). Secondly, the prevalence of anal HPV was also particularly high (#70%) and unique due to the high prevalence of HR-HPV (82.7%), its high genotypes diversity and the frequent (52%) multiple HR-HPV infections. Thirdly, the distribution of anal HPV in anal samples appeared unusual, the most prevalent genotypes being HPV-35, HPV-58, HPV-59 and HPV-31, while the more classical HPV-16 and HPV-18 were present only in a minority of samples (#25%), likely indicating possible regional clusterization in the diffusion of HR-HPV within the MSM community living in Bangui. Fourthly, HPV types included in the prophylactic Gardasil-9[®] vaccine were detected in the majority of HPV-positive anal samples (#70%) suggesting that the current 9-valent vaccine could be beneficial for the prevention of HPV-associated disease in this MSM community, although one-third of HPV anal infection would not be prevented. Finally, anal HSV-2 shedding was not associated with anal HPV shedding in our study population, in which the only negative modulatory cofactor for HPV anal carriage was behavioral, the insertive anal intercourse being relatively protective in comparison with receptive anal intercourse. Taken together, our observations indicate for the first time that the MSM community living in Bangui should be at very high-risk for HIV infection as well as HR-HPV anal infections, and strongly suggest that scaling up prevention strategies against HPV infection and related cancers adapted to this highly vulnerable MSM community should be urgently prioritized with innovative interventions.

Very high prevalence of HIV-1 was observed in the study MSM, suggesting that the HIV prevalence in the MSM community of Bangui could be high. This observation is reminiscent to similar reports on MSM living in sub-Saharan Africa. Studies conducted in sub-Saharan African countries in the last 10 years show that HIV prevalence among MSM is more than

5–18 times higher as compared to general population [37, 38]. In Tanzania, the prevalence of HIV in MSM was 17.4% and 3.7% among the general population [3]. Similarly, in Malawi, the prevalence of HIV was 21.4% in MSM and 6.1% in the general population [39]. In Kenya, the HIV prevalences in MSM ranged from 12.3% to 43.0% as compared to 6.1% in the general population [40]. In Ivory Coast, the prevalence of HIV in MSM was as high as 50% as compared to 3.2% in the general population [41].

The prevalence of anal HPV was particularly high (#70%) in this sample of MSM from the Bangui's community. The high prevalence of anal HR-HPV in our study MSM appeared quite similar to the prevalence reported in MSM living in South Africa and Nigeria, ranging from 58% to 72% [6, 7], but lower than the prevalence reported in young Black American MSM (87%) [42]. Other reports conducted outside Africa showed lower anal HR-HPV prevalence rates, ranging from 29% to 56% [43–46]. Previous reports have clearly demonstrated that the elevated risk for anal HPV in MSM is increased by HIV infection. Thus, anal HR-HPV infection was up to 4–10 times more frequent in MSM living in many countries outside Africa than in heterosexual men [47, 48]. Finally, our observations confirm that anal HPV constitutes a major infectious health concern in the MSM living in Bangui, highly escalated by HIV infections, and that each MSM community is characterized by local epidemiological specificities rendering necessary their research before intervention.

In our series, the distribution of anal HPV in anal samples appeared quite atypical, with HPV-35 being the predominant genotype. Furthermore, HPV-16 and HPV-18 were very poorly represented in the study population with the most prevalent 9-valent vaccine HR-HPV being HPV-58. This unusual distribution mirrors the previous observations by Nowak and colleagues, reporting that anal samples from MSM living in Nigeria harbored HPV-35 as the predominant genotype, HPV-16 and HPV-18 as minor genotypes, and HPV-58 as the most prevalent 9-valent vaccine genotype [7]. Interestingly, MSM infected by HPV-35 in our series and in MSM living in Nigeria [7], were all co-infected with HIV likely suggesting that HIV infection may play a role in the persistence of this unusual HPV type as a predominant genotype. Likewise, another recent study conducted in the United States of American highlighted the low proportion of HPV-16 and HPV-18 in anal samples from young black American MSM [42]. In contrast, anal HPV-16 and HPV-18 were the most prevalent in MSM living in South Africa, including a majority (67%) of mixed race/colored and white people and only (31%) one-third of black individuals [6]. Taken together, these observations suggest the possibility of a regional distribution in molecular epidemiology of HR-HPV within the diverse MSM communities inside the sub-Saharan African continent [49]. Thus, it is possible to hypothesize that anal cancers in certain black African MSM populations may be due to other HR-HPV rather than HPV-16 and HPV-18, which constitute the HR-HPV types involved in more than 89% of all anal cancers in MSM living in Western countries [50, 51]. Interestingly, others studies on HPV infection in women living in Nigeria have also reported HPV-35 as the predominantly isolated genotype [52,53]. In the Central African Republic, there is no data on the HPV type specific prevalence in the general population. It would be interesting to check whether the unusual HPV genotype distribution found in our Central African MSM series is similar to that in the female or general population. Further studies are nevertheless needed to determine the natural history and the burden of HPV-associated diseases in black African MSM in order to confirm our observations and to formulate effective and adapted HPV vaccine strategies towards young African MSM.

HPV types included in the prophylactic Gardasil-9[®] vaccine were detected in the majority of HPV-positive anal samples. Around 70% of all anal HPV-positive individuals harbored at least 1 of the 9-valent HPV vaccine genotypes and 45% of HPV-positive anal specimens contained multiple HPV vaccine types. High rates of 9-valent HPV vaccine types in anal canal of

MSM were previously reported in South Africa (57%) [6]. These observations indicate that MSM living in Bangui, as other MSM populations, constitute a key target population for HPV vaccination with the current prophylactic Gardasil-9[®] vaccine, which would potentially prevent most of HPV infections and associated anal diseases. However, anal HR-HPV not included in the prophylactic nonavalent vaccine, including the unusual HPV-35, were found in one-third of the study's MSM, indicating that the current HPV vaccine may be insufficient to prevent HPV-related diseases in a significant proportion of the MSM community living in Bangui. Thus, the guidelines on HPV immunization recommended in 2015 by the American Cancer Society (ACS), which integrate HPV vaccination up to 26 years for young MSM with the current two large spectrum HPV vaccines [54, 55], because HPV-16 and HPV-18 are mostly involved in HPV-associated anal cancer in Western countries [50, 51], may be poorly adapted to the MSM community living in the Central African Republic and other sub-Saharan African settings.

In vitro interactions between HPV and HSV-2 [16], in the context of high HSV-2 prevalence in sub-Saharan Africa [18], prompted us to evaluate the possible association between anal HSV-2 DNA shedding and anal HPV detection. The rate of anal HSV-2 DNA detection in our series (11.9%) was similar to that (10.6%) reported in a recent meta-analysis on MSM living in China [56]. However, no association between anal shedding of HSV-2 and HPV detection could be found.

Multiple HR-HPV was frequently detected in anal swabs from Bangui's MSM, mainly in HIV-infected individuals. Multiple HR-HPV in MSM is yet to be well documented [6, 21, 42, 51, 57]. High rates (91–94%) of multiple anal HPV infections with numerous different HPV genotypes ranging from 0 to 18 (mean, 4.8–5.0) were reported in HIV-positive MSM living outside Africa, such as North Canada and Thailand [58, 59]. In the present series, multiple HR-HPV infections were more frequently detected in HIV-infected than HIV-negative MSM and multiple anal HR-HPV infection was 15-times more frequent in HIV-positive than in HIV-negative MSM (univariate analysis). Indeed, high-risk sexual behavior, including exclusive sex with other men while being HIV-infected, constitutes a significant cofactor strongly associated with increased risk of multiple anal infections with HR-HPV genotypes [13–15]. In our study MSM population, MSM-exclusively showed 7-times more frequently receptive than insertive anal intercourse (88% versus 13%). Furthermore, MSM-exclusively were all HIV-positive (100%), thus more frequently infected by HIV than those having sex with both men and women (62%).

The principal negative modulatory cofactor for HPV anal carriage was behavioral, the MSM practicing condomless receptive anal intercourse being at more risk to be infected with HPV than those practicing insertive anal intercourse. Condomless receptive anal intercourse in MSM is well known to constitute the main sexual risk factor associated with HPV infection [6, 14, 15].

These findings are reminiscent to previous reports on HIV transmission demonstrating that MSM practicing receptive anal intercourse are at higher risk for HIV acquisition than their insertive partner [60, 61]. More generally, a receptive partner is more vulnerable to HPV infection than insertive partner in anal intercourse, as previously demonstrated in a recent study on 733 HIV-infected individuals (538 MSM, 195 heterosexual) that showed the prevalence, clearance and incidence of HPV infection was higher in the anal mucosa (73%, 30% and 36%, respectively) than in penile mucosa (26%, 56% and 17%, respectively) [62]. Indeed, the rectal mucosa, which consists of a single layer of epithelial cells, may break easily, exposing the epidermoid cells of the basal lamina, thus facilitating their infection by HPV [63]. Furthermore, the anal mucosa is relatively large and the rectal receptacle may retain pathogens [63, 64]. Circumcised men, as MSM living in Bangui, have keratinized external mucosa of the penis that provides strong epithelial barrier hampering HPV infection [63, 64].

Finally, HIV-positive MSM reported practicing condomless receptive oral sex were more frequent than HIV-negative MSM, and this sexual practice constituted a risk factor was strongly associated with having anal infection with any type of HPV and particularly HR-HPV. Hu and colleagues have previously reported in HIV-infected Chinese MSM that oral sex, even protected, is closely linked with anal HPV infection, suggesting that other high-risk practices of the sexual repertoire, yet unevaluated, may be associated [44]. Taken together, these observations are similar to a previous report in South Africa where the population of HIV-positive MSM-exclusively constituted a high-risk group accumulating several risky sexual behaviors and multiple anal HR-HPV infections [6]. In short, these observations emphasize the urgent need to implement adapted intervention strategies towards African MSM to reduce sexual risk behaviors. Intervention strategies such as counseling for HIV and sexual risk behaviors have been shown to reduce the incidence of HIV and STI among MSM [65–68]. Furthermore, scaling up prevention strategies against HR-HPV infections and associated cancers adapted for this at-risk vulnerable population should be prioritized. Targeted behavioral interventions may include promotion of consistent condom use [65–68], clinical examination with digital ano-rectal touching (“DARE”) which is an important tool for the early detection of precancerous lesions and anal cancer [69], medical male circumcision to reduce HIV incidence, as well as prevalence and persistence of anal HR-HPV infections [70], and finally HPV prophylactic vaccination of young MSM [55].

In conclusion, MSM community living in Bangui constitutes a very high risk population for both HIV infection and HR-HPV anal infections, and should urgently receive adapted STI and anal cancer screening and care.

Our study had some limitations. Indeed, the recruitment of participants from only the CNRIST/TAR of Bangui as well as the small sample size of our study population, may have introduced selection and information bias. Thus, the study participants may be not completely representative of the MSM community of the Central African Republic, especially regarding prevalences of HIV and anal HPV, and the genotypes distribution of anal HPV. Furthermore, some risk factors may have been underestimated in the statistical analyses.

Supporting information

S1 Data. Excel data sheet containing raw data on socio-demographic, behavioral characteristics, as well as the results of different biological analyzes carried out on MSM living in Bangui included in the study.
(XLSX)

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4. Efficacité d'un dispositif d'auto-prélèvement (voile vaginal) pour le dépistage des infections à HPV par PCR multiplex en temps réel chez des femmes adultes vivant en Afrique subsaharienne

Ce paragraphe est basé sur l'article intitulé "*Accuracy of Cervical Cancer Screening Using a Self-Collected Veil for HPV DNA Testing by Multiplex Real-Time PCR among Adult Women in sub-Saharan Africa*", soumis pour publication dans la revue scientifique "*BMC women's Health*" et actuellement en cours de révision. Il traite de l'évaluation d'un dispositif d'auto-prélèvement de sécrétions cervicovaginales pour la réalisation du dépistage moléculaire de l'infection à HPV par PCR multiplex en temps réel, au sein d'une population de femmes adultes vivant au Tchad.

Avant-propos.

Rationnel et objectif de l'étude. Le cancer du col utérin est causé par des infections génitales à Papillomavirus haut-risque (HR-HPV). En Afrique subsaharienne le cancer du col de l'utérus est en phase de devenir le premier cancer de la femme dans plusieurs pays (Mboumba Bouassa et al., 2017). Les programmes nationaux de lutte contre le cancer du col, basés sur les stratégies de prévention secondaire, ont du mal à fournir les résultats escomptés (Mboumba Bouassa et al., 2017). L'un des principaux freins à ces programmes est la faible couverture du dépistage. En effet, une grande partie des femmes africaines à risque pour le cancer du col n'a pas toujours accès aux structures de santé spécialisées où sont effectués les tests moléculaire et cytologique pour la détection de l'infection à HPV et les lésions précancéreuses. Il devient dès lors nécessaire d'adapter ces stratégies au contexte Africain. L'auto-prélèvement de sécrétions génitales pour la réalisation du dépistage moléculaire de l'infection à HPV et réalisé par les femmes elles-mêmes, sans assistance médicale, constitue une très bonne alternative susceptible d'augmenter l'efficacité des stratégies de prévention en améliorant la couverture du dépistage. L'étude transversale GYNAUTO-CHAD a comparé l'acceptabilité et la précision du diagnostic de l'infection à HPV réalisé à partir d'échantillons endocervicaux collecté par deux méthodes : un prélèvement réalisé à l'aide d'un écouvillon par un clinicien (Méthode de référence) et un prélèvement auto-administré par la patiente sans assistance médicale à l'aide d'un voile (V-Veil-Up Gyn Collection Device, V- Veil-Up Pharma Ltd., Nicosie, Chypre).

Méthodologie. Cinq des 10 districts de N'Djamena ont été choisis au hasard pour être inclus. Des éducateurs ont contacté des femmes adultes dans des églises communautaires et des mosquées ou des réseaux d'associations de femmes pour participer à l'enquête et se rendre au

dispensaire pour la santé sexuelle des femmes « La Renaissance Plus ». Un clinicien a procédé à un examen pelvien et obtenu un échantillon endocervical à l'aide d'un écouvillon floqué. Les sécrétions génitales ont également été obtenues par auto-collecte en utilisant le voile. Les échantillons prélevés par le clinicien et ceux recueillis par les femmes ont été testés par PCR multiplex en temps réel. L'acceptabilité des deux méthodes de collecte a été évaluée et la positivité du test a été comparée en évaluant la concordance, la sensibilité et la spécificité des méthodes.

Résultats. Au total, 253 femmes (d'âge moyen, 35,0 ans) ont été incluses de manière prospective. La prévalence de l'infection à HPV était de 22,9%, dont 68,9% de HR-HPV, avec une distribution inhabituelle des géotypes de HR-HPV et une prépondérance (environ 70%) des HR-HPV ciblés par le vaccin Gardasil-9®. L'auto-collecte génitale basée sur le voile a montré une acceptabilité, une faisabilité et une satisfaction élevées (96%). L'auto-collecte par voile était non-inférieure à la méthode de référence pour le test moléculaire HR-HPV, avec un « bon » accord entre les deux méthodes, une sensibilité élevée (95,0%; IC 95%: 88,3-100,0%) et une spécificité (88,2 %; IC95%: 83,9-92,6%). De manière remarquable, les taux de positivité en HPV et HR-HPV étaient significativement plus élevés (1,67 et 1,57 fois, respectivement) lorsqu'on utilisait des sécrétions génitales recueillies à partir d'un voile, par rapport aux sécrétions cervicales collectées par un clinicien.

Conclusions. Ces observations mettent en évidence l'importance de l'infection HR-HPV oncogène cervicale chez les femmes tchadiennes. L'auto-collecte des sécrétions génitales à l'aide du dispositif de prélèvement V-Veil-Up Gyn constitue un outil simple, hautement acceptable et puissant pour collecter les sécrétions génitales en vue de la réalisation de tests HPV pour des femmes vivant dans des zones rurales éloignées des structures de santé.

**Acceptability and Accuracy of Cervical Cancer Screening Using a Self-Collected
Veil
for HPV DNA Testing by Multiplex Real-Time PCR
among Adult Women in sub-Saharan Africa**

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Running head. Cervical HPV DNA detection by self-collected veil.

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Abstract

Background. Cervical cancer is caused by high risk-HPV (HR-HPV) genital infections. Self-collection of genital specimens and HPV DNA testing are methods increasing screening rates. The cross-sectional GYNAUTO-CHAD study compared the acceptability and HPV DNA diagnostic accuracy of clinician-collected endocervical sample with swab (as reference collection) and genital self-collection method with a veil (V-Veil-Up Gyn Collection Device, V-Veil-Up Pharma Ltd., Nicosia, Cyprus) in adult African women.

Methods. Five of the 10 districts of N'Djamena were randomly selected for inclusion. Peer educators contacted adult women in community churches and mosques or women association networks to participate to the survey and to come to the clinic for women's sexual health "La Renaissance Plus". A clinician performed a pelvic examination and obtained an endocervical specimen using flocked swab. Genital secretions were also obtained by self-collection using veil. Both clinician- and self-collected specimens were tested for HPV and HR-HPV DNA using multiplex real-time PCR. Acceptability of both collection methods was assessed; test positivity was compared by assessing methods agreement, sensitivity and specificity.

Results. A total of 253 women (mean age, 35.0 years) was prospectively enrolled. The prevalence of HPV infection was 22.9%, including 68.9% of HR-HPV, with unusual HR-HPV genotypes distribution and preponderance ($\approx 70\%$) of HR-HPV targeted by Gardasil-9® vaccine. Veil-based genital self-collection showed high acceptability (96%), feasibility and satisfaction. Self-collection by veil was non-inferior to clinician-based collection for HR-HPV DNA molecular testing, with "good" agreement between both methods, high sensitivity (95.0%; 95%CI: 88.3-100.0%) and specificity (88.2%; 95%CI: 83.9-92.6%). Remarkably, the rates of HPV DNA and HR-HPV DNA positivity were significantly higher (1.67- and 1.57-fold, respectively) when using veil-based collected genital secretions than clinician-collected cervical secretions by swab.

Conclusions. These observations highlight the unsuspected high burden of cervical oncogenic HR-HPV infection in Chadian women. Self-collection of genital secretions using the V-Veil-Up Gyn Collection Device constitutes a simple, highly acceptable and powerful tool to collect genital secretions for further molecular testing and screening of oncogenic HR-HPV that could be easily implemented in the national cervical cancer prevention program in Chad.

Key words: Cervical cancer; HPV detection; HPV genotyping; Multiplex real-time PCR; Self-collection; Clinician-collection; Veil; Sub-Saharan Africa

Background

Human papillomavirus (HPV) infection is the most common viral sexually transmitted infection (STI) worldwide and high risk-HPV (HR-HPV) genotypes are responsible for 5.2% of all cancers worldwide, 2.2% of cancers in developed countries and 7.7% of all cancers in developing countries [1-3]. In sub-Saharan Africa, cervical cancer associated with persistent cervical HR-HPV infection is the most common cancer in women in many countries, with more than 75,000 new cases and nearly 50,000 deaths registered each year [4, 5]. Increasing incidence of cervical cancer in Africa is counteracting the progress reached in the survival and the longevity of African women. Thus, the nearly 40% reduction in maternal mortality in Africa over 20 years [6, 7] is now paradoxically contemporary with the increase in female mortality related to cervical cancer in Africa, due to high prevalence of female genital carriage of HR-HPV and increased life expectancy [7]. According to the World Health Organization (WHO), cervical cancer will kill annually more than 443,000 women around the world by 2030 and 98% of these deaths will occur in developing countries and especially in Sub-Saharan Africa [5, 6]. Cervical cancer has become progressively one of the main public health challenges to overcome in sub-Saharan Africa since the recent decades [7].

Cervical cancer is a potentially preventable disease, including primary prevention with vaccination for women early before the first sexual intercourse to avoid further HR-HPV infection, and secondary prevention based on the early detection of HR-HPV infections by various techniques [7]. Many developed countries have significantly reduced the incidence of cervical cancer through expansive programs for early detection of HPV infection by molecular biology and cervical smear with Pap test [8]. However, cytology-based screening programs in low-resource countries have not been equally successful because of poor organization of screening programs, low coverage of the target population, and both inadequate insurance coverage and quality of screening tests [7]. The inaccessibility of specialized health structures and the difficulties to carry out Pap smear in the African context are among the main causes of the failure of cervical cancer prevention programs mainly based on Pap smear screening [4, 7]. Conversely, the detection of HR-HPV by molecular techniques has proved to be a good alternative to Pap smear in sub-Saharan Africa [7, 9]. In the African context, the "screen-and-treat" strategy combining visual inspection of the cervical mucosa after acetic acid staining and molecular tests for the detection of oncogenic HPV followed by cryotherapy treatment of HR-HPV-associated cervical dysplasia has proven to be effective for

screening and care in a very short time [7]. This approach significantly limits the number of visits to large specialized health centers [7].

In order to increase the coverage of screening programs in resource-limited countries, self-sampling of genital samples intended for molecular testing constitutes a promising alternative to Pap smear screening [10-16]. Self-sampling may be easily carried out individually by women at home without special medical qualification and special assistance [17, 18], and allows women to preserve their intimacy [12, 19, 20]. Molecular detection of HR-HPV using self-collected genital secretions (collected at home or at health care center) has proven to be nearly as sensitive as molecular screening performed on samples collected by clinician in specialized health care facility [11, 21-30]. In the African context, self-sampling of genital secretions was generally well accepted and easily feasible [11-13, 15, 16, 29], and it may furthermore facilitate the screening of cervical cancer in remote populations far from large health care centers [20, 31]. Finally, self-sampling may be especially valuable as an alternative method of cervical cancer screening as a method to enroll women who otherwise would not participate in population-based cervical cancer screening [17], and particularly in resources-constrained areas [9, 27].

The epidemiology of HPV infection is poorly known in Chad. Chad is a country of around 15 million people, including more than 3 million women aged more than 25 years [32, 33]. No significant progresses have been realized until now in cervical cancer prevention [34-36]. Only one pilot study assessing the feasibility of the cytology-based screening for cervical lesions in HIV-infected women has been conducted in Chad [35]. Mortier and colleagues found that HIV-infected Chadian women were at high-risk for low and high-grade cervical lesions, suggesting indirectly unsuspected high burden of cervical HPV infection in Chad [35]. Unfortunately, epidemiological data on the distribution of circulating HR-HPV genotypes in the general population in Chad are still always missing [37, 38].

The main objectives of the present study, so-called GYNAUTO-CHAD operational research program, were to assess the acceptability and feasibility of a novel genital veil (V-Veil-Up Gyn Collection Device, V-Veil-Up Pharma Ltd., Nicosia, Cyprus) in adult African women as a genital self-sampling device to collect cervicovaginal secretions. Furthermore, the study aims to evaluate the accuracy of veil-collected genital secretions to diagnose HPV infections by multiplex real-time PCR, as compared to reference clinician-collected genital specimens. Finally, the study was also conceived to provide data on the molecular epidemiology of female genital HPV infections in N'Djamena, the capital city of Chad.

Methods

Study design

The cross-sectional GYNAUTO-CHAD study compared the acceptability and HPV DNA diagnostic accuracy of a clinician-collected endocervical sample with swab and a cervicovaginal self-collection method with veil device in adult women living in N'Djamena, Chad, recruited from the community. The 2015 Standards for Reporting of Diagnostic Accuracy (STARD) guidelines were used for reporting the study [39, 40].

Enrolment and selection criteria

The capital city N'Djamena comprises 10 districts, which include a variable number of neighborhoods. Twenty-three sites in neighborhoods of 5 districts randomly selected out of 10 were further chosen for study inclusion [1st district : Farcha, Amsiné, Karkandjeri ; 3rd district : Gardolé, Ardep Djoumal, Kabalaye, Sabangali ; 6th district : Moursal, Paris-Congo ; 7th district : Ambata, Amtoukouin, Atrone, Boutalbagara, Chagoua, Dembé, Gassi, Habena, Kilwiti ; 9th district : Digagali, Gardolé, Ngueli, Tourkra, Walia]. In each inclusion site, peer educators contacted adult women in in community churches and mosques or women association networks during a one-month period and proposed them to be included in the GYNAUTO-CHAD study after an oral explanation and collective awareness sessions on the objectives of the survey, mainly focused on sensitization on cervical cancer and prevention strategies against cervical HPV acquisition. After oral consent, the selected women were invited, with paid transportation, to come to the clinic “La Renaissance Plus”, N'Djamena, which is one of the main settings for women’s sexual health in Chad, and to participate in the study. Childbearing-aged and older women living in N'Djamena regularly attend the clinic “La Renaissance Plus” for gynecological examinations and for obstetrical services.

The inclusion criteria were being a volunteer, having given signed informed consent, being aged between 25-65 years (consistent with current cervical cancer screening recommendations [7]), being sexually active, having no genital troubles at physical examination, being not menstruating, having no sexual intercourse for at least 48 hours (as recommended for HPV molecular testing in female genital secretions [41]) and having completed the questionnaire. Exclusion criteria included age less than 25 years and more than 65 years, having genital troubles, having menstruations, having recent sexual intercourse less

than 48 hours, not willing to participate to the study or to answer the face-to-face questionnaire to collect data.

Note that the menstrual cycle phases were not taken into account, since it has been previously demonstrated that they do not affect HPV detection in female genital secretions [42].

Clinical visit procedures and genital samples collection

The Figure 1 depicts the overview of the one-time clinical visit procedures of the GYNAUTO-CHAD study.

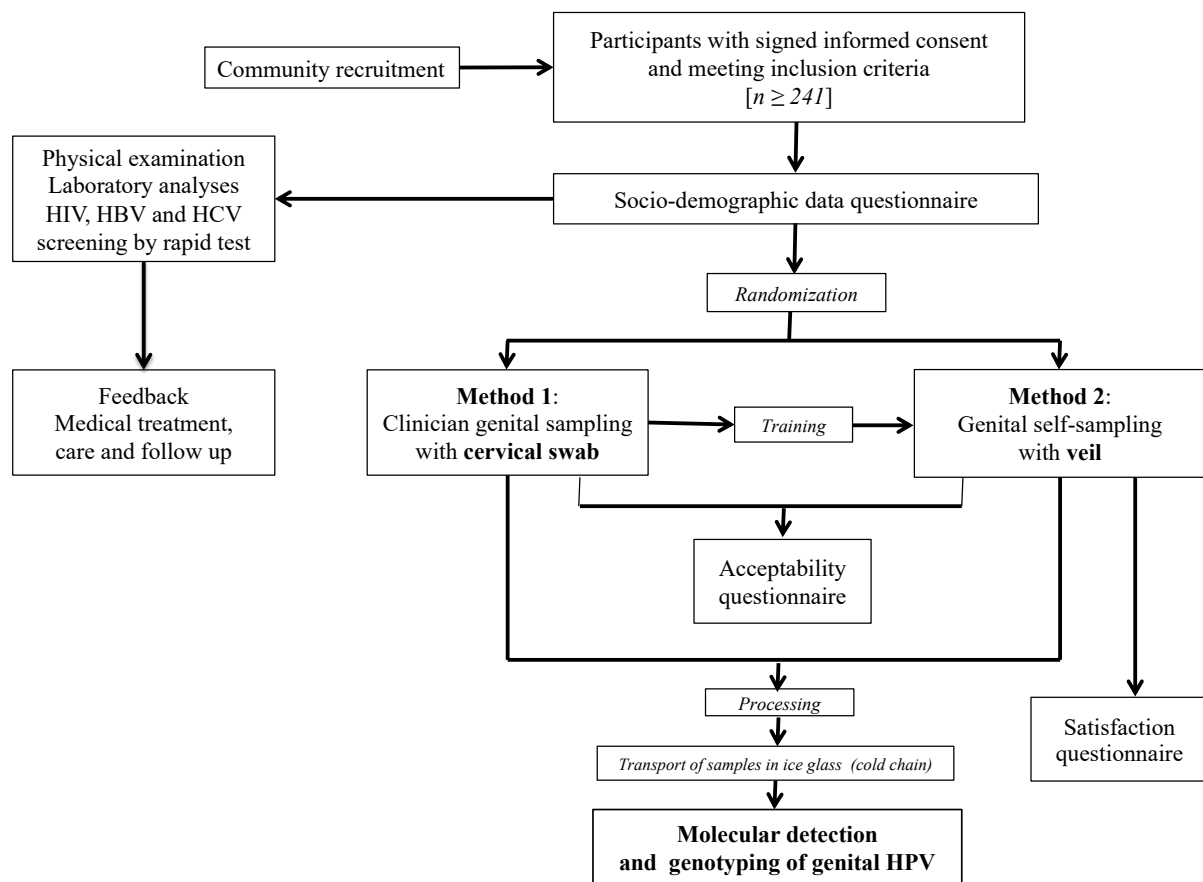


Figure 1. Flow diagram of the GYNAUTO-CHAD study. The GYNAUTO-CHAD study consisted in community-based recruitment of at least 261 adult women to be referred to the gynecologic clinic “La Renaissance Plus”, N’Djamena, Chad. The participants meeting the inclusion criteria were subjected to physical examination and care when needed, and tested for 3 chronic viral infections endemic in Chad (HIV, HBV and HCV) by capillary-based immunochromatographic rapid test, and filled in the face-to-face socio-demographic questionnaire. Afterwards, the participants were trained for self-sampling collection using the V-Veil-Up Gyn Collection Device (V-Veil-Up Pharma Ltd.) and randomly submitted to the sampling procedure with Method 1 (clinician-based collection of cervical secretions by swab, as gold standard) followed by the Method 2 (self-collection by veil) or inversely. The acceptability and satisfaction questionnaire were then administrated to the study women and collected samples were processed before molecular detection and genotyping of genital HPV infection.

Women eligible for the study were received by a medical staff (preferably nurse) who explained the progress of the step-by-step protocol and had them to sign the informed consent (Figure 1).

After having signed the informed consent form, the selected women benefited from free HIV and hepatitis B (HBV) and C (HCV) testing, by multiplex HIV/HCV/HBsAg immunochromatographic rapid test (Biosynex, Strasbourg, France) [43], clinical services including gynecological examination, family planning counseling, STIs diagnosis, laboratory analysis when necessary and appropriate treatment for those suffering from gynecologic disorders, HIV or other genital infections. All women received an information session on HIV and STIs.

At inclusion, a standardized interview was conducted at the clinic “La Renaissance Plus”, by experienced counselors, using a face-to-face questionnaire, to collect socio-demographic characteristics and behavioral data, including age, marital status, social occupation, education level, residence location in N’Djamena, history of STI, HIV status, birth control method, genital hygiene during menses, sexual behavioral characteristics such as the number of lifetime sexual partners and the age at first sexual intercourse, and assessment of knowledge regarding cervical cancer.

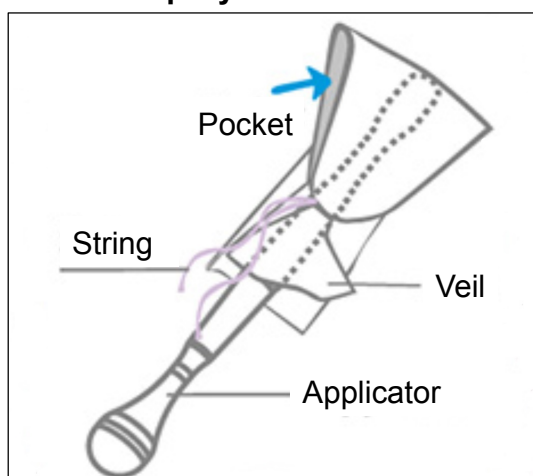
In order to eliminate any possible bias of sampling method and timing, the participants were further randomly selected to collect the genital secretions by clinician-based swab sampling first, followed by the veil-based self-sampling after the nurse-training, or by the veil-based self-sampling first followed by the clinician-based swab sampling. Thus, after completion of the socio-demographic questionnaire, all the biological specimen were sampled and processed in the following order: i) Samples specific for each patient for medical exams according to the medical prescription following the consultation; ii) Endocervical swab collected by a doctor [Method 1] or self-collection of genital secretions using the V-Veil-Up Gyn Collection Device [Method 2] according to the randomization.

The gold standard Method 1 was carried out by a doctor using a flocked swab (Copan Diagnostic Inc., California, USA). Briefly, after placing the speculum (without lubricant prior to insertion), the physician used the swab to perform cervical sampling by introducing it into the cervical canal and performing 5 rotations before being removed and immediately placed in its plastic container. The swab was then placed in the cold (ice pack).

For the self-collection Method 2, the study participant firstly received from a nurse a 15-minutes training on how to use the V-Veil-Up Gyn Collection Device for vaginal self-sampling (Figure 2A). After instructing the participant, the nurse leaved the sampling room and the participant then performed herself the self-sampling, without any help from the nurse.

The participant followed the instructions for use of the V-Veil-Up Gyn Collection Device (Figure 2B). Briefly, the study woman inserted the veil into her vagina, leaved it in-place for one hour; then removed it with the string, and returned it to the nurse. The study nurse did not witness veil insertion and removal. The nurse placed the veil impregnated with genital secretions into the dedicated collection box and closed it correctly with the cap. The veil collection box consisted of a 15 mL plastic box that contained 10 mL of phosphate-buffered saline (PBS) solution to prevent drying of the sample. The nurse verified that the PBS buffer completely submerged the veil and checked that the identification number in the label on the collection box corresponded effectively to the participant. The veil in its box was then placed in the cold (ice pack).

A. V-Veil-Up Gyn Collection device



B. Instructions for use (steps)

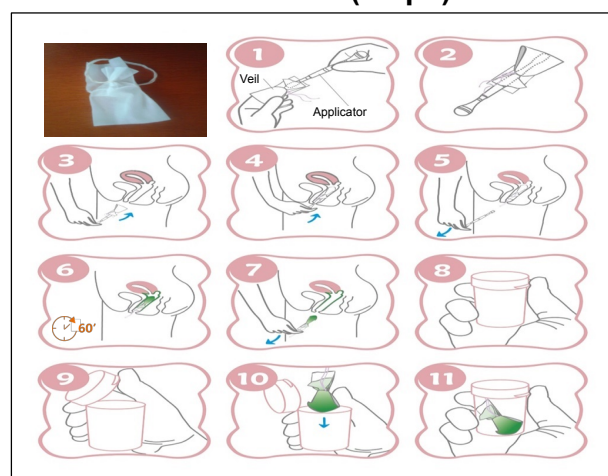


Figure 2. A. V-Veil-Up Gyn Collection Device (V-Veil-Up Pharma Ltd.). The V-Veil-Up Gyn Collection Device consists in a pocket (length: 75 mm, width: 45 mm, surface of contact: 6,75 cm²) in non-woven hydrophilic polyethylene having a capacity of absorption of 1.4 g of liquid, associated with a thread in cotton yarn (nm 16/4) and a transparent applicator (length: 120 mm, diameter: 10.8 mm) in low-density polyethylene (LD-PE). The plastic applicator makes possible to push the veil to the bottom of the vagina after its intrusion into the pocket, and the string allows the veil to be easily removed after use. The V-Veil-Up Gyn Collection Device is intended for the safe and non-invasive collection of cervicovaginal secretions from the vaginal canal. The device is conceived for self-sampling collection in privacy, and collected cervicovaginal fluid may be further gathered from the veil to be used for biological analysis. The V-Veil-Up Gyn Collection Device catches and retains safely and gently the genital secretions, thus harvesting cells, proteins and nucleic acids (DNA/RNA) from the vaginal canal. By its conception, the device does not absorb the liquids, that allowed full recovery of collected biological material, which is not trapped like in a vaginal tampon or sponge.

B. Successive steps of the instructions for use of V-Veil-Up Gyn Collection Device.

Components of the cervicovaginal secretions adhere to the veil in a collection process time lasting 60 minutes (Nota bene. The manufacturer recommends letting in place the collecting device for 30 to 60 minutes; in GYNAUTO-CHAD study, we arbitrarily chosed the maximum time of sampling, i.e. 60 minutes). Upon removal of the device from the vaginal canal, the sample veil is immediately protected with an opaque collection box that preserves the integrity of the genital secretions during transportation to a laboratory where downstream testing may be performed. A cap is placed on the collection box of the device to seal the unit, which is then ready for transportation to a laboratory. At least 30 minutes after taking a shower or washing the vagina must be expected to collect specimens using the V-Veil-Up Gyn Collection Device. In addition, in order to avoid sampling of male genital secretions, self-sampling should be carried

out after at least 2 days after unprotected sexual intercourse. In preparation for cervicovaginal collection, the contents of the device are placed on a clean and dry surface. The name or identification number of the woman as well as the time (date and hour) of sampling are tagged on a label sticker on the collection box. In a comfortable position, the V-Veil-Up Gyn Collection Device is taken in the dominant hand, hold the handle between the thumb and middle finger, with the index finger free above the base of the handle. With the other hand the skin folds of the large and small lips are hold open to open the vaginal canal. The veil is then aligned with the vaginal canal by pushing the applicator. The V-Veil-Up Gyn Collection Device is then gently pressed into the vaginal canal until the contact of the cervix. It is recommended not to urinate during the time of collection. After a period of 60 minutes, the collection is considered as complete, and the veil impregnated of cervicovaginal secretions is gently removed from the vaginal canal using the string. The sample veil should then not be touched, and kept clear of any other objects. Immediately after removal, the veil is placed into a 15 mL plastic box containing 10 mL of phosphate-buffered saline (PBS) solution to prevent drying of the sample, and is closed with his cap. The veil filled of secretions in its box is then placed in the cold (ice packs or crushed ice), and should be then send at best to the laboratory within 4 hours.

After returning the veil specimen, a second nurse administered acceptability and satisfaction questionnaires on the woman's experiences about the pelvic examination and clinician-based collection and the veil-based self-collection. To minimize bias, the study nurse who performed the pelvic examination was in another room and did not participate in the post-test questionnaire administration. The objectives of the acceptability questionnaire was to evaluate the study women's experiences related to the perception of care, comfort, privacy, embarrassment, or pain associated to each collection method. The satisfaction questionnaire consisted in questions regarding the ability to understand the instructions for use of the V-Veil-Up Gyn Collection Device and its component and to perform self-collection, including home collection, and also assessed difficulties encountered during veil-collection.

Genital samples processing

Each genital sample was transported in ice packs within an hour after collection to be stored at -80°C at the virology laboratory of the hôpital Général de Référence Nationale, N'Djamena, Chad. Swabs and veils were further transported in frozen ice packs to the virology laboratory of the hôpital Européen Georges Pompidou, Paris, France, for molecular analyses.

Nucleic acid extraction

DNA was extracted from the tip of swab specimens using the DNeasyBlood and Tissue kit (Qiagen, Hilden, Germany), as recommended by the manufacturer. After extraction, DNA was concentrated in 100 µL of the elution buffer provided in the extraction kit and stored at -80°C before HPV DNA detection and genotyping, as described previously [44].

Veil samples soaked with genital secretions within PBS buffer were carefully removed from their collection box and placed into a syringe to be drained by pulling the syringe's

plunger into a 15 mL tube. The whole genital secretions were then vigorously vortexed to homogenize the fluids and finally aliquoted in 1.5 mL cryotubes (Eppendorf, Hambourg, Germany) and store at -80°C before the nucleic acid extraction procedure. In order to avoid any contamination between different specimens, the working area was sterilized between the processing of each specimen and all the consumables including gloves, syringe, forceps were for single use and were immediately discarded together with the box container. Finally, the nucleic acid extraction procedure was carried out with the DNeasy Blood and Tissue kit (Qiagen), in 1 mL of the concentrated cervicovaginal veil-collected specimen and extracted DNA was placed in 100 µL of the elution buffer provided in the extraction kit and stored at -80°C before HPV DNA detection and genotyping.

HPV detection and genotyping

HPV detection and genotyping was performed on both the clinician- and self- collected specimens using the CE IVD-marked multiplex real-time PCR assay Anyplex™ II HPV28 (Seegene, Seoul, South Korea), as described previously [45]. The kit contains specific primers targeting 28 HPV, and is based on Seegene's proprietary DPO™ and MuDT™ technologies [46], which allow to avoid mismatch priming and to quantify each target in a single fluorescence channel, respectively. According to the HPV classification nomenclature provided by the International Agency for Research on Cancer (IARC) [47] Anyplex™ II HPV28 technology allows to detect 28 HPV genotypes in a single specimen, including 13 high-risk types (HR-HPV -16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68), 9 low-risk (LR) types (LR-HPV -6, -11, -40, -42, -43, -44,-53, -54 and -70) and then, 6 genotypes classified as possibly carcinogenic (HPV-26, -61, -66, -69, -73 and -82). Briefly, 5µL of swab- or veil- extracted DNA were added into two reaction mixtures (20 µL) containing each other, one of the primers sets A and B [45]. The DNA amplification and the genotyping process were carried out in 2 reactions performed on the CFX96™ real-time PCR instrument (Bio-Rad, Marnes-la-Coquette, France) [45]. The Anyplex™ II HPV28 genotyping test has been found to be suitable for HPV detection and genotyping in cervical secretions [45, 48-51]. Data recording and interpretation were automated with Seegene viewer software (Seegene), according to the manufacturer's instructions. A sample was considered positive for any HPV if containing any of the 28 types targeted by the Anyplex™ II HPV28 detection test; positive for multiple HPV when containing at least 2 types of the 28 HPV types included in genotypic test; HR-HPV positive and multiple HR-HPV positive when containing respectively at least 1 HR-HPV type and at least 2 high-risk types belonging to the 13 high-risk types targeted by the Anyplex™ II HPV28 detection test, irrespective of the presence of

LR-HPV. The virology laboratory was accredited in 2013 by the Comité Français d'Accréditation (COFRAC) according to the ISO 15189 norma for the biological markers "HPV detection" and "HPV genotyping".

Sample size

We hypothesized that Method 2 (self-sampling) would be non-inferior to Method 1 (flocked swab as gold standard), with a tolerated difference of Δ in the detection rate of HPV infections by molecular analysis between the two methods of collection. The requested minimum number (n) of subject to include was obtained by using Epi Info version 3.5.4 (CDC, Atlanta, USA), and by setting 95% confidence level, 80% statistical power, and considering estimated HPV prevalence of two methods in Chad. There are no data on the prevalence of genital HPV infections among women living in the Chad. In order to estimate the HPV DNA positivity in our study population, we used prevalences of genital HPV detection from comparable populations of women living in other Central African countries previously published in the literature, including 12.5% in Democratic Republic of the Congo [52], 18.5% and 34.0% in Cameroon [53, 54], and 22.2% in Rwanda [55]. Based on this assumption, we estimated the mean prevalence (P1) of HPV DNA test results to be 21.7% in the clinician-collected arm. We conducted a non-inferiority comparison with the hypothesis that the difference in HPV DNA positivity between the veil-based self-collection and clinician-collection methods would be less than 10%. With Δ of 10%, the requested minimum number (n) of subject to include was at least 241 participants.

Statistical analyses

Data was entered into an Excel database and analyzed using IBM® SPSS® Statistics 20 software (IBM, SPSS Inc, Armonk, New York, USA). Means and standard deviations (SD) were calculated for quantitative variables and proportions for categorical variables. The results were presented along with their 95% confidence interval (CI) using the Wilson score bounds for categorical variables. The overall prevalences of HPV DNA detection [any genotypes, HR-genotypes and HPV genotypes targeted by the 9-valent Gardasil-9® vaccine (Merck & Co. Inc., New Jersey, USA)] between the two collection methods were compared using the Mac Nemar's test for paired data. The Wilcoxon's test of paired data was used for comparison of the mean notes according to the Likert scale of acceptability of the two methods (veil-based self-collection versus swab-based clinician-collection). The agreement between the two collection methods was estimated by Cohen's κ coefficient, and the degree of

agreement was determined as ranked by Landlis and Koch [56]. Percent agreement corresponded to the observed proportion of identical results between veil-based self-collection compared to swab-based clinician-collection. Note that the acceptability of the clinician-based collection by swab and that of the veil self-collection were assessed using an arbitrary quantitative Likert scale [57] based on four different scale ranging from 1 (most difficult), 2 (difficult), 3 (easy) to 4 (= very easy or comfortable). Similarly, the satisfaction regarding the veil self-collection method was assessed using another arbitrary quantitative Likert scale based on four different scale ranging from 1 (less favorable), 2 (moderately favorable), 3 (favorable) to 4 (= most favorable). The mean and standard deviation for Likert scale data were calculated for each acceptability and satisfaction item using face-to-face questionnaires. The clinician-collected HPV DNA test results were used as the reference standard to estimate the sensitivity and specificity, with corresponding 95%CI, of the veil-collection method.

Results

Characteristics of study population

From June to October 2017, 271 women from the 23 inclusion sites accepted to participate to the study. The geographical distribution of the participants was as follow: 1st district (43, 15.9%): Farcha (n=15), Amsiné (n=13), Karkandjeri (n=15); 3rd district (54, 19.9%): Gardolé (n=12), Ardep Djoumal (n=19), Kabalaye (n=10), Sabangali (n=13); 6th district (47, 17.4%): Moursal (n=23), Paris-Congo (n=24); 7th district (61, 22.5%): Ambata (n=5), Amtoukouin (n=6), Atrone (n=9), Boutalbagara (n=5), Chagoua (n=7), Dembé (n=8), Gassi (n=9), Habena (n=9), Kilwiti (n=3); and the 9th district (66, 24.3%): Digangali (n=10), Gardolé (n=7), Ngueli (n=11), Toukra (n=12), Walia (n=26) (Figure 3).

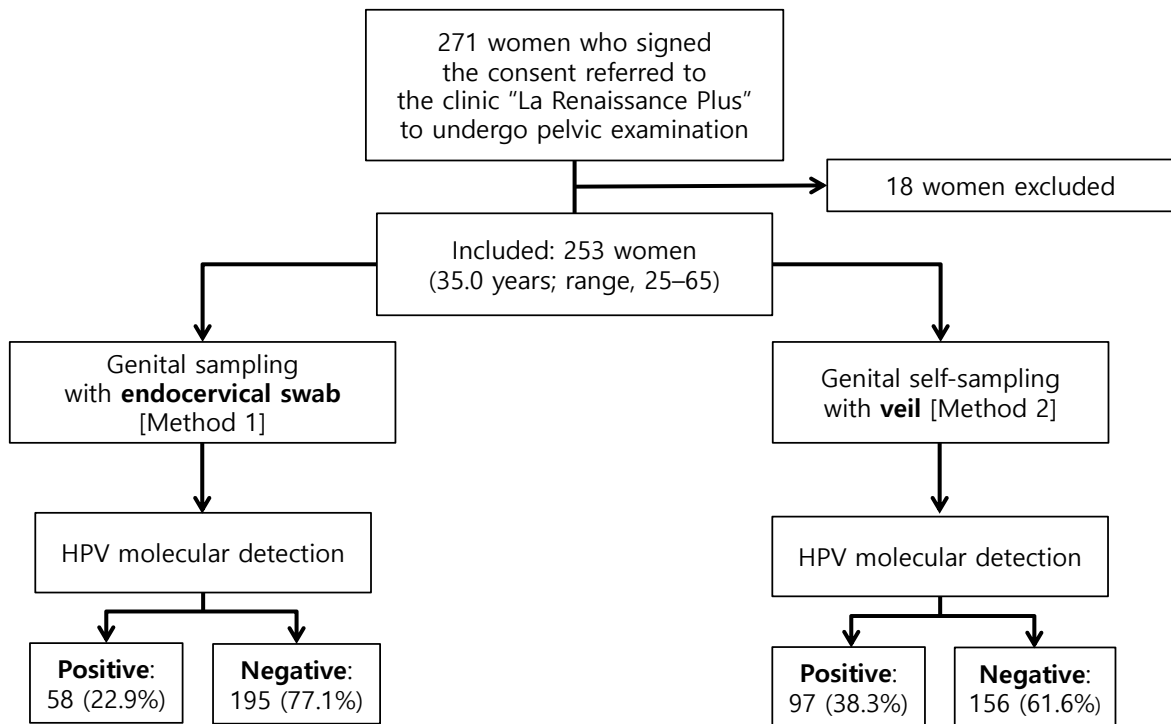


Figure 3. Flow diagram of study recruitment, specimen collection, and HPV test results by multiplex real-time PCR.

After physical examination, 18 women were excluded because of genital troubles (vaginal discharge: 5; suspicion of STI: 3; genital bleeding: 5; sexual intercourse less than 2 days: 5). Finally, a total of 253 women (mean age, 35.0 years; range, 25–65) referred to the clinic “La Renaissance Plus” were consecutively and prospectively included in the study (Figure 3). Their socio-demographic characteristics, past history of STIs, sexual behavior, contraception and practices of feminine hygiene during menstruation and genital toilet are summarized in the Table 1.

Using multiplex HIV/HCV/HBsAg rapid test, 9 study women (3.5%; 95% CI: 1.3–5.8) were infected by HIV-1, 19 (7.5%; 95% CI: 4.3-10.8) by HBV (positivity for HBsAg) and 8 (3.2%; 95% CI: 1.1-5.3) were seropositive for HCV.

Most women (31.6%; 95% CI: 25.9–37.4) were young, aged from 25 to 29 years, engaged in life couple with a male partner (78.3%; 95% CI: 73.2–83.3), with a relatively high education level (32.1%; 95% CI: 26.3–37.7 and 30.4%; 95% CI: 24.7–36.1, in high school level and university, respectively); but most of them were unemployed (54.2%; 95% CI: 48.1–60.3). The majority of study women (82.2%; 95% CI: 77.5–86.9) reported having only one regular sexual partner in their life, while about 20% reported to have had up to 5 different sexual partners. Generally, the study women began sexual activity at 16 to 20 years (56.2%; 95% CI: 50.1–62.3), whereas some of them (13.8%; 95% CI: 9.6–18.1) started their sexual life earlier, before the age of 16 years. The vast majority of women (74.4%) did not take any

birth control methods. Concerning the feminine hygiene during menstruation, most women (90.3%) were using sanitary napkins, while a minority (13.0%) used commercially available tampons. Genital (vulva or vagina) toilet was the rule, including post-coital toilet with water and finger in 90.3%. Finally, none of the women included in the GYNAUTO-CHAD study had ever been screened for cervical cancer and nor vaccinated against HPV infection.

Acceptability of collection methods

Participants reported feeling much better cared for during the veil-based self-collection (mean note of 3.1 according to the Likert scale of acceptability) compared to swab-based clinician-collection (mean note of 1.4; $P < 0.02$) and also more in privacy handled during self-collection (mean note of 3.1) compared to clinician-collection (mean note of 1.4; $P < 0.005$) (Table 2). There were no other significant differences in embarrassment, discomfort or genital pain between the two collection methods (Table 2). When asked to choose one collection method, 243 (96.0%) of study women responded that they would prefer the self-collection method. Furthermore, most participants (237; 89.7%) reported that they would be willing to perform veil-collection at home and bring the specimen with them to clinic.

Table 2. Acceptability of veil-based self-sampling using the V-Veil-Up Gyn Collection Device (V-Veil-Up Pharma Ltd.) compared to swab-based clinician-collection for HPV DNA testing among 253 study women living in N'Djamena, Chad.

Acceptability items*	Veil-based self-collection [mean (SD)]	Swab-based clinician-collection [mean (SD)]	<i>P</i> -value**
How well cared for did you feel?	3.1 (0.8)	1.4 (0.5)	0.011
How well was your privacy handled during the test?	3.1 (0.5)	1.4 (0.5)	0.003
Did you feel embarrassed?	3.3 (1.2)	3.1 (1.4)	0.835
Did the test cause you any genital discomfort?	3.1 (1.3)	3.1 (1.2)	1.000
Did the test cause you any genital pain?	2.9 (1.4)	2.5 (1.4)	0.700

Satisfaction of self-sampling using the V-Veil-Up Gyn Collection Device

The results of the face-to-face satisfaction questionnaire regarding veil-based self-sampling using the V-Veil-Up Gyn Collection Device are shown in the Table 3. In addition, most women (231; 91.3%) reported that the instructions for use written in French were easy to read and to understand, while the verbal explanations on how to use the collection device showed higher mean note according to the Likert scale of satisfaction (written versus oral explanation: 2.9/4 and 3.6/4, respectively). A significant number of participants (76; 30.0%) reported difficulties in correctly interpreting schemas. The large majority of women (245; 96.8%) were

able to recognize correctly the component's device, with high notes (3.6/4). In addition, the veil was generally (243; 97.6%) correctly placed with the applicator and removed with the string. Difficulties on understanding how to place the veil deep within the vaginal cavity were frequently encountered in one-third of participants (86; 33.9%) with a low mean note of 0.9/4. All items concerning the general satisfaction of the V-Veil-Up Gyn Collection Device showed high mean notes from 2.9 to 3.3. Discomfort when carrying the veil concerned only urges to urinate. Genital pain when placing or wearing the veil was reported in a minority of women (10; 3.9%), all being more than 45 years-old. Finally, only 12 (4.7%) women reported some difficulties with performing the self-collection.

Table 3. Satisfaction questionnaire regarding veil-based self-sampling using the V-Veil-Up Gyn Collection Device (V-Veil-Up Pharma Ltd.) among 253 study women living in N'Djamena, Chad.

Variables*	Veil-based self-collection [mean (SD)]	95%CI
Understanding of instruction for use		
Instruction for use in French language	2.9 (1.1)	[2.7-3.0]
Verbal explanation of instruction for use	3.6 (0.5)	[3.5-3.7]
Anatomic sketches	2.4 (1.1)	[2.2-2.5]
Understanding of component's device		
Device has three components (veil ; applicator and string)	3.6 (0.4)	[3.6-3.7]
Veil includes pocket for drug or cream	3.6 (0.5)	[3.6-3.7]
Correct use of the veil		
Applicator to be placed in the pocket	3.7 (0.5)	[3.6-3.7]
String to be used to remove the veil after use	3.8 (0.4)	[3.8-3.7]
Place the veil deep within the vaginal cavity	0.9 (0.1)	[0.9-1.0]
General satisfaction		
Keeping the veil into the vagina for 60 minutes	3.1 (0.8)	[3.0-3.2]
Removing the veil impregnated with genital secretions with the string	3.3 (0.7)	[3.2-3.4]
Comfort when carrying the veil	3.3 (1.2)	[3.2-3.5]
Genital pain when placing or wearing the veil	2.9 (1.4)	[2.7-3.1]
General opinion about veil-based self-sampling	3.1 (0.5)	[3.0-3.1]

* The scale of satisfaction was asses by a Likert scale ranging from 1 (= less favorable) to 4 (= most favorable); the results are mean \pm 1 standard deviation (SD); 95% confidence intervals (CI) are given in brackets.

Prevalences of HPV detection and genotypes distribution by collection methods

All 253 study participants had paired clinician-collected and self-collected specimens obtained for laboratory testing. All secretions from swab and veil specimens were positive for the ubiquitous β -globin gene, used as internal control of cell sampling of the Anyplex™ II

HPV28 kit. Results from each of the collection method are presented in Tables 4, 5 and 6 and in Figures 4, 5 and 6.

Table 4. Two-by-two tables of cervicovaginal specimens self-collected using the V-Veil-Up Gyn Collection Device (V-Veil-Up Pharma Ltd.) compared to clinician-collected endocervical swab specimens for the detection by multiplex real-time PCR of any HPV genotypes, HR-HPV genotypes and IPV genotypes targeted by the 9-valent Gardasil-9® vaccine (Merck & Co. Inc.).

		Any HPV genotypes		HR-HPV genotypes		9-valent vaccine HPV genotypes				
		Clinician-collected swab specimen		Clinician-collected swab specimen		Clinician-collected swab specimen				
		Positive (n=58)	Negative (n=195)	Positive (n=40)	Negative (n=213)	Positive (n=29)	Negative (n=224)			
Veil-based self-collected specimen	Positive (N [*] =97)	56	41	Positive (N ^{***} =63)	38	25	Positive (N [§] =48)	28	20	
	Negative (N ^{**} =156)	2	154	Negative (N ^{****} =190)	2	188	Negative (N [‡] =205)	1	204	
		Estimate	95% CI			Estimate	95% CI			
Sensitivity (%)		96.5	91.8-100.0			95.0	88.3-100.0	96.5		89.8-100.0
Specificity (%)		78.9	73.3-84.7			88.2	83.9-92.6	91.1		87.3-94.8
Agreement (%)		83.0%				89.3%		91.7%		
Cohen's κ coefficient [¶]		0.611				0.675		0.682		

^{*} : Total number of veil based-self collected specimens positive for any HPV;

^{**} : Total number of based-self collected specimens negative for any HPV;

^{***} : Total number of veil based-self collected specimens positive for HR-HPV;

^{****} : Total number of veil based-self collected specimens negative for HR-HPV;

[‡] : Total number of veil based-self collected specimens positive for any HPV genotypes targeted by the 9-valent Gardasil-9® vaccine (Merck & Co. Inc.) (HPV -6, -11, -16, -18, -31, -33, -45, -52 and -58);

[‡] : Total number of veil based-self collected specimens negative for any HPV genotypes targeted by the 9-valent Gardasil-9® vaccine;

[¶] : The Cohen's κ coefficient was interpreted according the Landis and Koch scale [56]: For κ value 0, the agreement is considered to be less than what would be expected by chance; for κ values 0.01–0.20, only a “slight agreement” is present; for κ values 0.21–0.40, the agreement is considered to be “fair”; for κ values 0.41–0.60, the agreement is said to be “moderate”; for κ values 0.61–0.80, the agreement is considered “good”; and finally, for κ values 0.81–0.99, the agreement is said to be “almost perfect”.

IPV: Human papillomavirus; HR-HPV: high-risk human papillomavirus.

Of the clinician-collected specimens included in the analysis, 58 women were positive for genital HPV DNA giving a total HPV prevalence of 22.9% (95% CI: 17.8–28.1), with 68.9% (40/58; 95% CI: 57.1-80.8) harboring cervical HR-HPV infection, providing a total

HR-HPV prevalence of 15.8% (95% CI: 11.3-20.3), as shown in the Table 1. The whole distribution of HPV genotypes in HPV-DNA positive cervical samples is detailed in the Figure 4. The Gardasil-9® vaccine HR-HPV type 58 was the predominant genotype (7/58; 12.1%), followed by the HR-HPV types 31, 35 and 56 and the LR-HPV types 42 and 44 with a prevalence of 10.3% (6/58). The 9-valent vaccine HR-HPV types 16, 45 and 52 and also the LR-HPV types 53 and 70 were present [prevalence of 8.6% (5/58)]. These HPV genotypes were followed by the HR-HPV types 18 and 51, 59 and 68 and the LR-HPV types 6 and 54 and finally the possibly oncogenic HPV types 73 and 82 with a prevalence of 6.8% (4/58). The HR-HPV-39 was present only in 3 women (5.2%) and none of the HPV positive samples was simultaneously positive for HPV-16 and HPV-18.

Of the veil-based self-collected specimens included in the analysis, 97 women showed genital shedding of HPV DNA that represented an overall HPV prevalence of 38.3% (95% CI: 32.4-44.3). Among these HPV positive women, 64.9% [(63/97); 95% CI: 55.45-74.4] were positive for HR-HPV genotypes giving a total HR-HPV prevalence of 24.9% (95% CI: 19.6-30.2) (Table 1). The whole distribution of HPV genotypes in HPV-DNA positive cervical samples collected by veil is detailed in the Figure 4. The distribution of HPV genotypes in the HPV DNA-positive women revealed that the LR-HPV genotype 42 (13/97; 13.4%) was the predominant genotype, followed by the HPV54 (12/97; 12.4%), HPV70 (11/97; 11.3%). The Gardasil-9® vaccine HR-HPV type 58, 52 and 31 were the predominant HR-HPV genotypes (9/97; 9.3%), followed by HPV16 (8/97; 8.2%), HPV18, HPV35, HPV45 and HPV68 (7/97; 7.2%), HPV39 (5/97; 5.2%) and finally HPV33 (2/97; 2.1%).

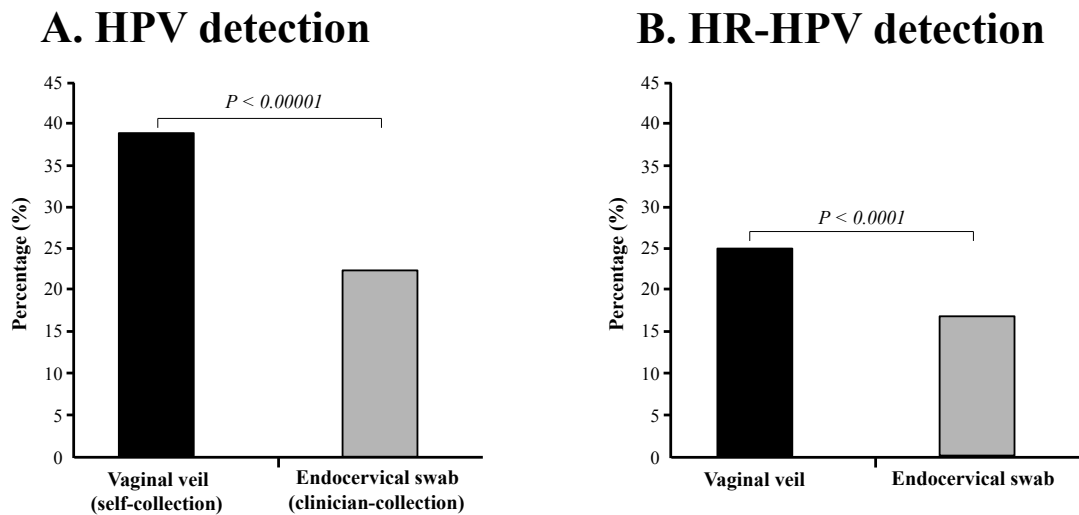


Figure 4. Percentages of detection by multiplex real-time PCR assay Anyplex™ II HPV28. HPV DNA (A) and HR-HPV DNA (B) in paired genital secretions obtained by gold standard clinician-collected endocervical swab and by self-collection using the V-Veil-Up Gyn Collection Device (V-Veil-Up Pharma Ltd.) among 253 study women living in N’Djamena, Chad. P-values of the comparison between the two collection methods using the Mac Nemar’s test for paired data are indicate in italic.

The percent agreements between the two collection methods to detect any HPV, HR-HPV and 9-valent vaccine HPV genotypes were 83.0%, 89.3% and 91.7%, respectively, and all Cohen's κ coefficients were between 0.61 to 0.80, demonstrating “good” agreement [56] (Table 4).

Using clinician-collected swab as the reference collection method, the sensitivities and specificities of the self-collected veil to detect HPV, HR-HPV and 9-valent vaccine HPV genotypes were 96.5% (95% CI: 91.8 – 100.0%) and 78.9% (95% CI: 73.3 – 84.7%), 95.0% (95% CI: 88.3 – 100.0%) and 88.2% (95% CI: 83.9 – 92.6%), and 96.5% (95% CI: 89.8 – 100.0%) and 91.1% (95% CI: 87.3 – 94.8%), respectively (Table 4).

Overall, the percentage of test positivity for HPV DNA was 1.67-fold higher in self-collected specimens than in clinician-collected specimens (38.3% versus 22.9%; P-value < 0.00001) (Figure 4A). The percentage of test positivity for HR-HPV DNA was 1.57-fold higher in self-collected specimens than in clinician-collected specimens (24.9% versus 15.8%; P-value < 0.0001) (Figure 4B). When considering the distribution of the 28 HPV genotypes detected by multiplex real-time PCR assay Anyplex™ II HPV28 detected in paired genital specimens, all genotypes but two (HPV-59 and HPV-82), were more frequently detected by self-collection using the V-Veil-Up Gyn Collection Device than by clinician-collected endocervical swab (Figure 5).

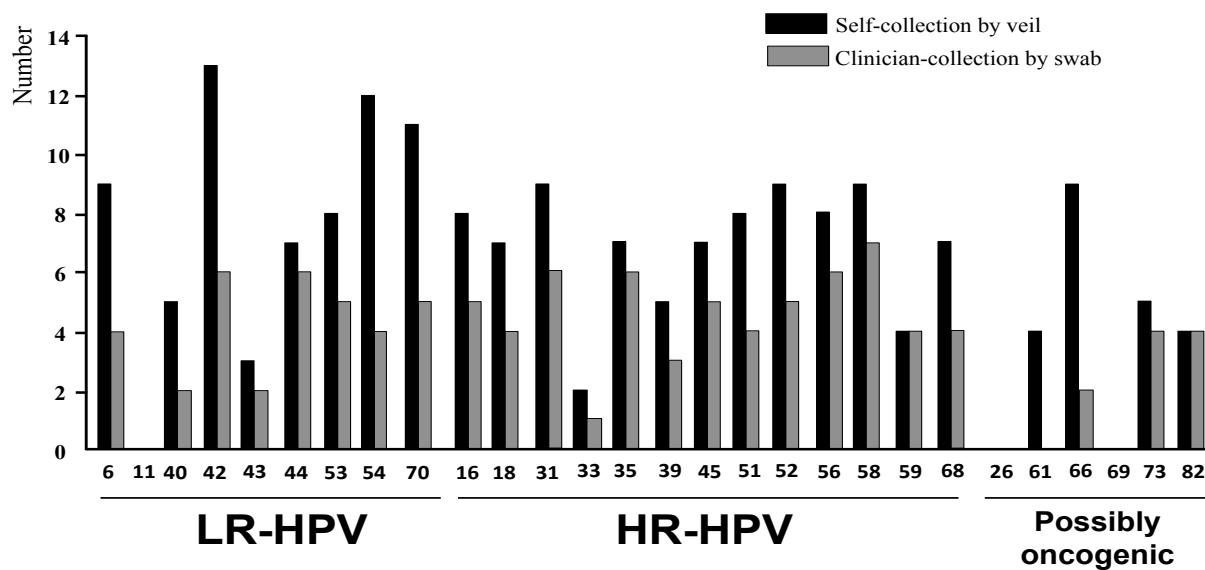


Figure 5. Distribution of the 28 HPV genotypes detected by multiplex real-time PCR assay Anyplex™ II HPV28. The HPV DNA detection was carried out in paired genital secretions obtained by clinician-collected endocervical swab and by self-collection using the V-Veil-Up Gyn Collection Device (V-Veil-Up Pharma Ltd.) among 253 study women living in N’Djamena, Chad. LR-HPV: Low risk- human papillomavirus; HR-HPV: High-risk human papillomavirus. The 28 HPV genotypes detected by the Anyplex™ II HPV28 kit include 9 low-risk types (LR-HPV), 13 high-risk types (HR-HPV) and 6 genotypes classified as possibly carcinogenic. The HPV genotypes -6, -11, -16, -18, -31, -33, -45, -52 and -58 which are targeted by the 9-valent Gardasil-9® vaccine (Merck & Co. Inc.) are highlighted in grey.

Among the 253 study participants, the mean numbers (± 1 SD) of HPV and HR-HPV detected when using the self-collected veil [1.84 ± 0.96 (range, 1-10) and 1.43 ± 0.64 (range, 1-5), respectively] were similar to those obtained when using the swab-collection [1.79 ± 1.14 (range, 1-9) and 1.5 ± 0.75 (range: 1-5), respectively].

The differential efficiency of detecting HPV and HR-HPV between the two collection methods was better evidenced when considering paired results by genotype. Thus, the correspondence between the 7,084 (=253 x 28) results obtained from the 253 paired swab-collected and veil-collected genital specimens to detect the 28 HPV genotypes included in Anyplex™ II HPV28 kit was further analyzed, genotype by genotype, and depicted in Table 5. The use of self-collection by veil allowed detecting 101 (84+17) additional HPV of all types by reference to the use of swab in 89 (35.2%) participants, while the use of swab allowed detecting only 7 additional HPV in 7 participants (2.8%) by comparison to the use of veil. When considering only the correspondence between the 3,289 (=253 x 13) paired results for oncogenic HR-HPV (Table 6), the use of veil allowed detecting 38 (34+4) additional HR-

HPV in 35 (13.8%) study women, while the use of swab allowed detecting only 3 additional HPV in 3 participants (1.2%) by comparison to the use of veil.

Table 6. Correspondence between the 3,289 (= 253 x 13) results obtained from the 253 paired swab-collected and veil-collected genital specimens to detect the 13 HR-HPV genotypes included in Anyplex™ II HPV28 kit (Seegene).

Swab	Veil	HR-HPV genotypes													Total
		16	18	31	33	35	39	45	51	52	56	58	59	68	
Negative	Negative	Number of cases per genotype													3,195
Positive	Positive with same genotype*	4	4	6	0	6	3	5	4	5	6	7	4	3	57
Positive	Positive with different genotypes** ^a	0	0	0	1 ^b	0	0	0	0	0	0	0	0	0	1
Negative	Positive	4	3	3	2	1	2	2	4	4	3	2	0	4	34
Positive	Negative	1	0	0	1	0	0	0	0	0	0	0	0	1	3

* *i.e.*, positivity using veil for the same HR-HPV genotype using swab;

** *i.e.*, positivity using veil for at least one another HR-HPV genotype using swab;

^aA total of 4 additional HR-HPV genotypes were detected using veil collection;

^bAdditional HR-HPV genotype detected using veil collection was HR-HPV -31, -51, -59 and -68.

NA: Not attributable; HPV: Human papillomavirus; HR-HPV: high-risk human papillomavirus.

The Figure 6 depicts some relevant examples of paired results obtained by clinician-collected endocervical swab and self-collected veil using the multiplex real-time PCR assay Anyplex™ II HPV28, showing the capacity of the veil to allow the detection of oncogenic HR-HPV that could not be detected from the paired swab specimens.

Specimen	HPV genotypes																										
	LR-HPV										HR-HPV										Possibly oncogenic						
	6	11	40	42	43	44	53	54	70	16	18	31	33	35	39	45	51	52	56	58	59	68	26	61	66	69	73
#004																											
Veil				+		+	+		+					+	+			+	+							+	+
Swab				+		+	+		+					+	+			+	+							+	+
#006																											
Veil				+				+	+																		
Swab																											
#104																											
Veil				+															+	+							
Swab																				+	+						
#108																											
Veil				+																							+
Swab																											
#127																											
Veil	+			+	+				+				+						+								
Swab																											+
#147																											
Veil									+					+													
Swab														+													
#173																											
Veil																											+
Swab																											+
#188																											
Veil																											+
Swab																											+
#199																											
Veil																											+
Swab																											+
#210																											
Veil																											+
Swab																											+
#257																											
Veil	+								+																		+
Swab	+																										+

Figure 6. Relevant examples of paired results obtained by gold standard clinician-collected endocervical swab and self-collected veil. Participants #006, #108, #199 and #257 were negative by clinician-collected swab, whereas they were positive by paired self-collected veil, with supplementary detection by veil of oncogenic HR-HPV, including HPV-16 in #006 and #199, HPV-68 in #108, and HPV-31, HPV-51 and HPV-68 in #257. Participants #004, #104, #127, #147, #173, #188 and #210 were positive by clinician-collected swab, and paired self-collected veil allowed supplementary detection of several oncogenic HR-HPV (HPV-18 in #188; HPV-31 in #210; HPV-33 in #127, HPV-39 in #147, HPV-52 in #004 and #104; HPV-68 in #173). Interestingly, all positive HR-HPV detections by clinician-collected swab were also detected by self-collected veil. Positive result for a given genotype is indicated by cross; negative result by white box. All swab and veil specimen were positive for β -globin internal control of the Anyplex™ II HPV28 kit (not shown).

Finally, genital infections with multiple HPV genotypes were more frequently detected using the veil collection device than swab collection [43.3% (42/97); 95% CI: 33.4-53.2 versus 27.6% (16/58); 95% CI: 16.1–39.1; P < 0.001], while the mean number of HR-HPV detected using positive genital secretions collected by veil was quite similar to that of cervical secretions collected by swab [1.7 HR-HPV genotypes (range, 1 to 5) versus 2.3 HR-HPV (range, 1 to 5)].

Discussion

The present study depicts for the first time the molecular epidemiology on the distribution of cervical HPV infection in adult women more than 25 years living in N’Djamena, the capital city of Chad. Unexpected high rates of cervical shedding of HPV and HR-HPV, 22.9% and 15.8%, respectively, were observed with unusual HR-HPV genotypes

distribution and the preponderance of cervical HR-HPV targeted by the Gardasil-9® vaccine, making the basis of the introduction of prophylactic vaccine against HPV in Chad. Furthermore, the acceptability and HPV DNA diagnostic accuracy of a novel genital veil (V-Veil-Up Gyn Collection Device) was assessed as female genital self-sampling device to collect cervicovaginal secretions. Interestingly, all specimens collected by the veil were found positive for the ubiquitous β -globin gene, demonstrating that they contained cellular DNA, which made HPV detection possible by molecular testing. The results showed high acceptability (96%), feasibility and satisfaction of the veil-based genital self-collection, which was non-inferior to clinician-based collection as reference for HPV DNA molecular testing, with “good” agreement between the two collection methods, high sensitivity of 95.0% and specificity of 88.2%. Outstandingly, the rates of HPV DNA and HR-HPV DNA positivities were significantly higher when using veil-based collected genital secretions than clinician-collected cervical secretions by swab. The self-collection by veil allows detecting 12.7-fold more additional oncogenic HR-HPV in 1 of 8 (12.5%) participants than the detection allowed by the swab-based collection, likely originating from non-cervical areas of the vaginal cavity, including vaginal cul-de-sacs, vaginal walls and vulva. Taken together, our observations highlight the unsuspected high burden of cervical HR-HPV infection in adult women more than 25 years living in Chad, with a high rate of preventable Gardasil-9® vaccine genotypes. Furthermore, veil-based self-collection of genital secretions appears a convenient tool to collect in a gentle way genital secretions for accurate molecular HPV detection and genotyping that could be easily implemented in the cervical cancer prevention program in Chad.

Based on clinician collection by swab as reference method to collect cervical secretions, more than one-fifth (22.9%) study women were shedding cervical HPV, which was mostly constituted of cervical HR-HPV (68.9%) and frequently due to multiple HPV genotypes (26.3%). Such high prevalence of HR-HPV appears notably higher as compared to those commonly reported in unscreened African women aged 25 years and older, with cervical HR-HPV infection rates generally not exceeding half of the HPV-positive women [7]. To our knowledge, high prevalences of cervical HR-HPV infection similar to that reported in the present series have yet been reported in other sub-Saharan African countries where HR-HPV prevalences ranged from 60.4% in Nigeria [58], to 67.9% and 68.5% in South-Africa [59, 60]. Different prevalences of cervical HPV in asymptomatic African women between studies, despite being conducted on the same continent and on quite similar populations, may be due to several factors, including genital sampling methods, HPV molecular testing tools as well as behavioral and socio-demographic factors [7].

Based on swab gold standard collection, the majority ($\approx 70\%$) of women infected by cervical HR-HPV showed high-risk genotypes covered by the 9-valent Gardasil-9[®] vaccine, with the HR-HPV-58 being the predominant genotype, followed by HPV-16, HPV-31, HPV-45, HPV-52 and HPV-18. Most previous studies conducted in sub-Saharan African countries depict a wide heterogeneity in the distribution of the main HR-HPV in women [52-55, 58-72]. The predominance of cervical HR-HPV-58 genotype in Chadian women of unknown cytology was never reported in other series from sub-Saharan Africa, and may constitute a particularity of the molecular epidemiology of HR-HPV in women living in Chad. The preponderance of cervical HR-HPV targeted by the Gardasil-9[®] vaccine in asymptomatic women living in Chad was commonly observed in women with normal cytology and in those with high-grade intraepithelial lesions in other sub-Saharan African countries [63]. Our study results on molecular epidemiology of HR-HPV are finally of paramount importance because they allow evaluating the predictive efficiency of vaccination against HPV with the Gardasil-9[®] vaccine in young Chadian girls.

Our observations highlight that women living in Chad form a neglected high-risk group for cervical HR-HPV infection and consequently for cervical cancer. These findings constitute the first report on the epidemiological burden of HPV infection in Chad. The very high prevalence of cervical HR-HPV in adult women clearly demonstrates that cervical HR-HPV infection in Chad constitutes a major public health problem, which remains largely unsuspected. Therefore, there is an urgent need for implementing a cervical cancer prevention program in Chad, as recommended by the WHO [73]. According to Mortier and colleagues, the cytology-based cervical cancer screening in women in Chad is feasible with low cost and easy to interpret visual technics; and could be integrated in existing healthcare structures [35]. Indeed, for these women carrying cervical HR-HPV infection, only secondary prevention with regular screening for precancerous lesions by cytology and the monitoring of the viral persistence by HPV molecular testing, remains the only alternative to prevent the disease progression into invasive cervical cancer. However, in the context of Chad, a very low-income country, there is a serious lack of pathologist specialists, thereby making conventional cytology not suitable and reinforcing on the other hand the great necessity to implement HPV DNA testing with molecular technologies [7]. Indeed, HPV DNA testing constitutes an alternative to cytology for cervical cancer screening, which is furthermore highly sensitive and reproducible [7]. HPV DNA molecular testing could promote the “screen-and-treat” strategy recommended by the WHO to prevent cervical cancer in developing countries [73], thus allowing to maximize the medical support in a single visit and avoiding the loss of women positive for HR-HPV.

Taking into account that most adult Chadian women are living in remote rural areas, or far away of adequate healthcare facilities, self-collection of genital specimen carried out at home by women themselves could represent a relevant alternative allowing increasing the coverage of screening when coupled with adapted HPV DNA testing by molecular biology [7]. In the GYNAUTO-CHAD study, we have had the opportunity to evaluate the acceptability and HPV DNA diagnostic accuracy of a novel genital veil (V-Veil-Up Gyn Collection Device) as female genital self-sampling device to collect cervicovaginal secretions. Veil-based self-collection proved to be a particularly well acceptable method easily collecting genital secretions within our cohort of community-recruited adult Chadian women. Thus, the veil was in the vast majority of participants (97.6%) correctly placed with the applicator and removed with the string, without any difficulties. When asked to choose one collection method, the vast majority (96.0%) of study women responded that they would prefer the self-collection method, demonstrating high acceptability of the genital sampling using the V-Veil-Up sampler device. Although most women (91.3%) reported that the instructions for use in French were easy to read and to understand, with correct recognizing of the component's device, the verbal explanations by the nurse on how to use the collection device were better appreciated, particularly to correctly interpret the schemas of the instructions for use, and how placing the veil deep within the vaginal cavity. These observations are in keeping with the frequent ignorance of female genital anatomy in study participants (not shown), who were not generally taking any birth control method, and were not using tampons for feminine hygiene during menstruation. However, in practice, the manipulation of the veil was almost correct, perhaps in relationship with the very frequent usage of genital toilet in study women. Thus, while the majority of participants were not already familiar with using tampons (only 13% in our study population), the genital manipulations during veil-based self-collection were easy to carry out, which might lead to greater preference for the veil much over than other unfamiliar methods, such as a brush or a swab. These findings are reminiscent to the high acceptability of the use of vaginal tampons for self-collection reported in African women living in South Africa [12, 29]. These observations suggest that a large proportion of African women might actually prefer self-collection methods not necessitating good knowledge of the female genital anatomy to other self-collection methods such as brush or swab, for which the women must specifically target their cervix. Furthermore, the answers of study participants demonstrated high satisfaction of the V-Veil-Up Gyn Collection Device. Thus, very few women reported difficulties when performing the veil-based collection and nearly all women reported positive experiences with collection. Only a minority (4.7%) of participants reported some difficulties with performing the self-collection, most frequently genital pain when placing or wearing the

veil in a minority of women (3.9%), all being more than 45 years-old, likely because of vaginal dryness in women being in the menopause period.

Otherwise, our observations also point the potential interest of using a supervised self-collection strategy among African women, in which oral counselling processes are aided at all times by a healthcare or non-healthcare professional as a counsellor to understand the instructions for use, the genital anatomy, and provide counselling with a very high rate of acceptability and satisfaction of self-collection. The evidence of high acceptability for supervised strategies was previously reported for another self-collection of capillary blood or saliva during HIV self-testing, especially in resource-constrained settings [74]. Other advantages of veil are that it is inexpensive and easily accessible. Finally, providing options for self-collection based upon women's preferences is likely to increase screening coverage, and our data suggest that veil is an acceptable option.

The percent agreements between clinician-based collection and veil-based self-collection to detect any HPV, HR-HPV and 9-valent vaccine HPV genotypes were above 80%, and all Cohen's κ coefficients were between 0.61 to 0.80 demonstrating "good" agreement between collection methods [56]. Although it exists no data on the performance of self-collected specimens by veil for HPV DNA testing for which to compare our results, several previous reports evaluating the use of self-collection by vaginal tampons and vaginal lavages for HPV DNA or HR-HPV mRNA may be useful to contextualize our results, since tampons and lavage as well as veil do not target particularly the cervix but are the reflect of the whole secretions of the vaginal cavity [75]. Thus, our findings of agreement between the two collection methods with the kappa-statistic are relatively similar to previous reports evaluating HR-HPV DNA detection by self-collection using vaginal tampons with clinician-collection as reference, with Cohen's κ coefficients ranging from 0.49 [76], 0.55 [77], 0.50 [11], 0.63 [42], 0.70 [78], 0.75 [77] to 0.76 [79] or vaginal lavage with Cohen's κ coefficients ranging from 0.47 [17], 0.53 [17], 0.64 [80], 0.65 [81], 0.71 [82] to 0.78 [81], thus indicating "moderate" to "good" agreements [56]. Similarly, the performances of HR-HPV mRNA testing using self-collected vaginal tampons by reference to clinician-collected specimens, with Cohen's κ coefficient of 0.54 indicating "moderate" agreement [29], were slightly below to the agreement of the veil.

Out of 40 HR-HPV-positive clinician-collected specimens, 38 veil-collected specimens were also positive for HR-HPV, corresponding to a sensitivity of 95.0%. Using vaginal tampon, Adamson and colleagues reported much lower sensitivity of 77.4% of vaginal tampons to detect HR-HPV mRNA by reference to clinician collection by swab [29]. A wide range of sensitivities of tampon collection using clinician-collection as the reference

were reported to detect HR-HPV DNA, ranging from 59% to 94% [11, 20, 22, 77, 83-86] and always lower than the sensitivity of the veil observed in our hands. Similarly, the reported sensitivities of vaginal lavage to detect HR-HPV ranged from 88% to 90% [81]. It remains unclear whether these differences are due to different duration of collection time, different order of specimen collection, or whether they reflect the differences in testing methods. One reason for the lower sensitivity of the tampon-based collection might be due to sampling location. The clinician-collected specimen, obtained directly from the cervix, preferentially collects cervical cells in the transformation zone, whereas the tampon-method provides a mix of cells from both the cervix and vagina, and therefore might not collect enough cells from the transformation zone [75]. In contrast, the high sensitivity with the veil to detect cervical HR-HPV demonstrates that the veil likely retains significant amount cells originating from the cervix and more than a simple vaginal tampon. It is possible that extending the time of holding the veil might increase the sensitivity, but it also might lead to decreased acceptability [20]. Finally, it is important to note that the reported sensitivities of self-sampling by vaginal tampons in addition with our own sensitivity with the veil are only for molecular detection of HPV, since no pathological data were recorded to predict dysplastic or pre-invasive lesions such as CIN 2+.

Likewise, of the 213 clinician-collected specimens negative for HR-HPV DNA, 188 veil-collected specimens were also negative for HR-HPV DNA, corresponding to a specificity of 88.2%. The specificity of the veil is of the same order to those previously reported for self-collection to detect cervical HR-HPV DNA by vaginal tampons, ranging from 80% to 92% [11, 20, 77, 83, 84], but higher than that previously reported for self-collection by tampons to detect cervical HR-HPV mRNA (77.7%) [29]. The difference in sampling location between the clinician-based collection using swab targeting the endocervix and the self-collection by veil or tampons collecting global cervicovaginal secretions might explained the higher rate of positive results by veil or tampons than by swab, since the veil as well as vaginal tampons might have picked up vulvovaginal HPV infections, which do not necessarily coincide with cervical infections [75, 87].

Because clinical management and research usually depend on single-point detection of HPV, it is important to use the collection method the most capable to detect HPV by molecular biology. The cumulative presence of HPV in female genital tract is always greater than its point prevalence, suggesting that single-point sampling is less than 100% sensitive [42]. Otherwise, the vaginal epithelium represents a much greater surface area than the cervical epithelium and as such offers a greater number of potentially HPV-infected cells to collect. Thus, the self-sampling approach using veil might not only sample the cervix, but it

will also sample the vaginal epithelium, with a potentially greater likelihood of detecting HPV. Indeed, vaginal epitheliums together with the cervix epithelium provides a potentially higher number of HPV-infected cells than the cervix alone. Remarkably, in the present study, the percentages of test positivity for HPV DNA and HR-HPV DNA were 1.67-fold and 1.57-fold higher, respectively, in self-collected specimens by veil than in clinician-collected specimens. Furthermore, when considering the distribution of the 28 HPV genotypes detected by multiplex real-time PCR assay Anyplex™ II HPV28 in paired genital specimens, the vast majority (92.8%) of genotypes were more frequently detected by self-collection using the V-Veil-Up Gyn Collection Device than by clinician-collected endocervical swab. In more than one-third (35.2%) of participants, the self-collection by veil allowed detecting nearly 14.4 (101/7)-fold more additional HPV (not detected by swab) than those detected using the clinician-collection by swab. Likewise, the use of veil allowed detecting 12.6 (38/3)-fold more additional HR-HPV in 1 of 8 (13.8%) participants than the use of swab. These findings suggest that the veil is able significantly releasing genital cells for HPV molecular testing, likely better than vaginal tampon. Indeed, cell clusters embedded in a rayon-covered cotton core tampon are not easily separated from the sampling device [41]. Our observations also demonstrate the high capacity of the veil to allow the detection of oncogenic HR-HPV present within the vaginal cavity that could not be detected from the paired swab specimens. Finally, the veil was more efficient to detect oncogenic HR-HPV in whole cervicovaginal secretions than the swab used to collect cervical secretions. Indeed, the veil collects all types of cervicovaginal secretions of the vaginal cavity, not exclusively the cervical secretions, and thus allows detecting non-cervical HPV. Non-cervical HPV are a priori located at non-cervical vaginal areas (vaginal cul-de-sacs, vaginal walls and vulva). Another possibility may be also to consider that cervicovaginal HPV could also come from the male sexual partner, as previously envisaged [41]. Indeed, recent unprotected vaginal intercourse might affect HPV detection because false-positive tests could result from detecting another person's HPV DNA, or false-negative tests could occur because vaginal penetration could mechanically remove HPV-infected cells. However, we had verified as an exclusion criterion that participants had not had sexual intercourse for at least 48 hours, as recommended for HPV molecular testing in female genital secretions to exclude the risk to catch semen-associated HPV or male-derived exfoliated HPV-infected epithelial cells [41, 42]. Nevertheless, the detection of HR-HPV in vaginal secretions constitutes strong biological evidence that the woman is exposed to oncogenic HPV, which is the basis of molecular HPV screening. Women screened positive for HR-HPV by veil should then be referred for new cervical molecular testing or cervical Pap smear. Indeed, it is well known that an HPV-positive result, regardless of the cytology result,

may cause anxiety, stress and concern [88], making mandatory the triage of women found HPV-positive using veil-collected vaginal secretions

Study limitations

Our study had several strengths. First, we tried to limit the selection bias of the study population in order to make this survey as much representative as possible of the female population in Chad. Thus, the study inclusions were initially carried out using a random sampling of districts in N'Djamena, with further selection of neighborhoods inclusion sites. Study women were referred to clinic “La Renaissance Plus”, N'Djamena, although they were not patients from the clinic, avoiding the obvious bias of recruitment by health care facilities. Moreover, the prevalences for HIV-1, HBV and HCV in study population was in accordance with the high endemicity of these three major chronic viral infections in Chad [89, 90]. Second, we recruited and screened a large sample of community-recruited women who represent a high-risk primary screening population. Third, both collection methods were performed sequentially on the same day, allowing for direct comparison of the samples collected. Fourth, we were able to assess both the acceptability as well as the performance of the veil-collection method for HPV DNA testing. However, our study has limitations. Thus, the participants could be not completely representative of the adult women community of Chad, especially regarding the prevalences of HIV and cervical HR-HPV, and the genotypes distribution of cervical HPV. Another limitation is that we were not able to directly evaluate which collection method could be the most favorable to further care HPV-positive women. Finally, we did not perform any pathological evaluation, including Pap smear or cervical biopsies on study participants and do not know the true disease status of these women.

Conclusions

Our observations highlight the unsuspected high burden of cervical oncogenic HR-HPV infection in Chadian women, and point the potential risk of further development of HPV-associated cervical precancerous and neoplastic lesions in a large proportion of women in Chad. Cervical cancer, its diagnosis and prevention, is thus one of the most important public health challenges that Chad has to face in a near future. Self-collection of genital secretions using the V-Veil-Up Gyn Collection Device constitutes a simple, highly acceptable and

powerful tool to collect genital secretions for further molecular testing and screening of oncogenic HR-HPV that could be easily implemented in the national cervical cancer prevention program in Chad. In regions of the world where access to care is limited due to socioeconomic reasons or clinician-collected samples may be limited due to personal and/or sociocultural concerns, self-collection method by veil may provide a way to extend screening to an underserved population.

List of abbreviations

COFRAC: Comité Français d'Accréditation; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HIV: human immunodeficiency virus; HPV: Human papillomavirus; HR-HPV; High risk-HPV; LR-HPV; Low risk-HPV; STI: Sexually transmitted infection; WHO: World Health Organization

Declarations

Ethical approval and consent to participate

The study was formally approved by the Scientific Committee of the Faculty of Health Sciences of the University of N'Djamena, constituting the National Ethical Committee. All included women gave their informed signed consent to participate to the study. For each included woman, the record of the consent to participate to the study was documented on each questionnaire. This consent procedure was formally approved by the Ethical Committee. All individual results of HPV detection and genotyping as well as HIV, HBV and HCV serologies were given to each study participant, and women harboring cervical HR-HPV were further retained at the clinic “La Renaissance Plus”, screened for cervical lesions and women showing positive cervical cytology were cared. Furthermore, the study results have been in extenso reported to health authorities of Chad during the national congress of gynecologists and midwives, held from 13 to 17 of November 2018 in the Centre d'Etudes et de Formation pour le Développement (CEFOD), N'Djamena, Chad.

Consent for publications

Not applicable.

Availability of data and materials

The datasets analyzed during the current study is available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ZAN, DS, RSMB, DK and LB have conceived and designed the research; DS and AMM carried out the ethical issues; RSMB, MM, LR, MMT and DV carried out the experiments; ZAN, RSMB and STW performed statistical analyses; ZAN, RSMB, STW and HP analyzed the results; ZAN, RBMB, CA, HP, DV and LB drafted the manuscript.

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B. Caractérisation de la réponse immunitaire humorale induite à la suite d'une infection à Papillomavirus humains

1. Caractérisation de la réponse immunitaire IgG génitale et sérique contre les génotypes de HPV appartenant aux groupes $\alpha 7$ et $\alpha 9$ chez des femmes adultes non-vaccinées non-éligibles pour la vaccination HPV selon les recommandations vaccinales actuelles

Ce paragraphe traite de la caractérisation de la réponse immunitaire humorale acquise après une infection à HPV chez des femmes immigrées originaires d'Afrique sub-Saharienne, fortement infectées par le VIH et les HR-HPV et vivant en France. Cette partie repose principalement sur l'article intitulé "*Serum and cervicovaginal IgG immune responses against $\alpha 7$ and $\alpha 9$ human papillomaviruses (HPV) in non-vaccinated women at risk for cervical cancer: Implication for catch-up prophylactic HPV vaccination*". Cet article a été soumis pour une publication dans le journal scientifique "*Public Library Of Sciences (Plos One)*" et est actuellement en cours d'analyse.

Avant-propos.

Rationnel et objectifs de l'étude. L'infection à papillomavirus humain (HPV) est l'infection virale sexuellement transmissible (IST) la plus répandue dans le monde. Les génotypes à haut risque (HR) -HPV sont responsables de 5,2% de tous les cancers dans le monde et de 7,7% de tous les cancers dans les pays en développement (Scheurer et al., 2005; Parkin et al., 2006; Mboumba Bouassa et al., 2018). Parmi les treize HP-HPV à haut risque (-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59 et -68) (Bouvard et al., 2009), HPV-16 et HPV-18 sont à l'origine de près de 70% des cancers du col utérin chez les femmes du monde entier (de Sanjose et al., 2010). La plupart des types de HR-HPV génitaux sont regroupés dans les espèces $\alpha 7$ (HPV-18, -45 et -68) et $\alpha 9$ (HPV-16, -31, -33, -35, -52 et -58) (de Villiers et al., 2004).

En Afrique subsaharienne, le cancer du col utérin associé à une infection persistante à HR-HPV est actuellement le premier cancer féminin dans de nombreux pays (De Vuyst et al., 2013; Ferlay et al., 2015). Selon l'Organisation mondiale de la santé (OMS), le cancer du col de l'utérus fera chaque année environ un demi-million de décès chez les femmes au cours de la prochaine décennie, principalement en Afrique subsaharienne, où l'épidémie de VIH et d'autres facteurs de risque aggravent la situation sanitaire liée au cancer (Ferlay et al., 2015;

OMS, 2015). Ainsi, le cancer du col utérin est progressivement devenu l'un des principaux défis de santé publique à surmonter en Afrique subsaharienne (Mboumba Bouassa et al., 2017).

L'une des principales stratégies contre le cancer du col utérin est la prévention primaire contre l'infection par les HPV par la vaccination des jeunes filles (Mboumba Bouassa et al., 2017). Actuellement, trois vaccins prophylactiques anti-HPV à base de VLP ont été mis au point et sont actuellement utilisés pour la prévention de l'infection à HPV et les maladies associées. C'est le cas du dernier vaccin HPV homologué, le vaccin nonavalent Gardasil-9® de Merck & Co. Inc. (Kenilworth, NJ, États-Unis) contenant les VLP de HPV-6, HPV-11 et deux VLP du groupe $\alpha 7$ (HPV-18 et HPV-45) et cinq du groupe $\alpha 9$ (HPV-16, -31, -33, -52 et -58) (Harper & DeMars 2017; Pinto et al., 2018). Les vaccins prophylactiques contre l'infection à HPV ont été largement évalués dans le cadre d'essais cliniques de grande envergure, comprenant des populations d'origines diverses et appartenant à des groupes d'âge différents; et ont démontré une bonne sécurité et une très grande efficacité à conférer une protection solide et durable contre l'infection à HPV et les maladies associées (Luckett & Feldman, 2016; Pinto et al., 2018; Ruiz-Sternberg et al., 2018; St Laurent et al., 2018; Chen et al., 2019; de Sanjosé et al., 2019; MacIntyre et al., 2019; Mavundza et al., 2019; Toh et al., 2019; Zhu et al., 2019). Selon les recommandations actuelles de l'OMS (OMS 2009; OMS 2014), les femmes adultes sexuellement actives de plus de 19 ans, qui ont probablement déjà été exposées aux HPV, ne sont plus éligibles pour la vaccination contre les HPV. Leur seule autre option existante, demeure la stratégie de prévention secondaire utilisant le dépistage moléculaire régulier de l'infection à HPV et la cytologie pour détecter et traiter précocement les lésions précancéreuses cervicales associées aux HPV (Mboumba Bouassa et al., 2017).

Au cours des années 2010, les flux migratoires africains ont envoyé environ 500.000 immigrants chaque année vers l'Europe (Flahaux & De Haas 2016). Environ 300.000 femmes immigrées originaires d'Afrique subsaharienne vivent en France (INSEE 2015). La plupart des immigrées africaines de première génération arrivent en Europe au début de leur vie adulte et la plupart d'entre elles arrivent déjà sexuellement actives (Insee 2015; Flahaux & De Haas 2016). Les femmes africaines immigrées de première génération vivant en Europe pourraient avoir un profil infectieux reflétant l'épidémiologie de leur pays d'origine où l'infection cervicale à HR-HPV est très répandue (Bruni et al., 2010) et exacerbée par la synergie dite « *syndémique* » jouée par l'épidémie de VIH et d'autres IST (McCloskey et al., 2017). Les femmes immigrées originaires d'Afrique subsaharienne et vivant en Europe ont une prévalence élevée d'infection génitale par le HPV, avec au moins deux fois plus d'infection par HR-HPV que la population féminine européenne (Chironna et al., 2010;

Tornesello et al., 2011). De plus, les femmes d'origine immigrée montrent un nombre inférieur d'initiation et d'achèvement du vaccin anti-HPV (van der Wal et al., 2005; Bruno et al., 2014; Grandahl et al., 2017; Møller et al., 2018) et elles sont moins dépistées pour le cancer du col utérin au cours de leur vie que les femmes nées en Europe (Grillo et al., 2012). Ainsi, les femmes adultes africaines immigrées de première génération vivant en Europe, en particulier celles infectées par le VIH, constituent un groupe très risqué pour le cancer du col de l'utérus.

La vaccination HPV de rattrapage chez les immigrantes de première génération s'avère être un défi de taille en termes de santé publique. En effet, les indications restreintes pour la vaccination anti-HPV chez les jeunes filles (jusqu'à 19 ans) et l'accès limité au dépistage cytologique ou moléculaire du col utérin entravent le succès global de la prévention du cancer du col utérin chez cette population vulnérable (Bosch et al., 2016; Bosch et al., 2018; Stern & Roden, 2019). La vaccination avec les vaccins prophylactiques a montré une protection de près de 67 à 90% contre l'incidence et la persistance de l'infection HPV ainsi que la survenue de lésions précancéreuses de haut grade, plus de 7 ans après la vaccination, chez les femmes de plus de 25 ans (Wheeler et al., 2009; Einstein et al., 2014; Skinner et al., 2014; Wheeler et al., 2016), même chez les femmes présentant des signes sérologiques d'exposition antérieure aux HPV (Olsson et al., 2009; Castellsagué et al., 2011), contestant ainsi les recommandations actuellement restreintes en matière de vaccination contre l'infection à HPV (Wheeler et al., 2009; Couto et al., 2014; Einstein et al., 2014; Skinner et al., 2014; Schiller et al., 2016; Wheeler et al., 2016). En outre, des essais cliniques en cours, tels que l'étude HPV-FASTER, suggèrent déjà qu'élargir les indications du vaccin anti-HPV chez les femmes âgées de plus de 30 ans en association avec un plus grand accès au dépistage primaire moléculaire du HPV pourrait être bénéfique pour prévenir le cancer du col de l'utérus (Bosch et al., 2016; Bosch et al., 2018; Stern & Roden, 2019). Cependant, il n'est toujours pas clair si un vaccin de rattrapage chez les femmes immigrées trop âgées au moment de l'introduction du vaccin serait bénéfique. Depuis 2006, le Comité consultatif sur les pratiques d'immunisation (ACIP) des États-Unis a recommandé la vaccination de rattrapage contre les HPV aux femmes de moins de 26 ans (Meites et al., 2019). Notez qu'en juin 2019, l'ACIP a recommandé la vaccination de rattrapage contre les HPV pour toutes les personnes de moins de 26 ans. L'ACIP n'a pas recommandé la vaccination de rattrapage à tous les adultes âgés de 27 à 45 ans, mais a reconnu que certaines personnes qui ne sont pas suffisamment vaccinées pourraient être à risque de contracter une nouvelle infection à HPV et pourraient bénéficier de la vaccination dans cette tranche d'âge. Par conséquent, l'ACIP a recommandé que les

décisions cliniques soient partagées concernant la vaccination potentielle chez ces personnes (Meites et al., 2019).

Ainsi, le but de cette étude était de traiter de manière immunologique le problème de la vaccination de rattrapage contre l'infection à HPV chez les immigrées africaines de première génération vivant en France, qui constituent une population vulnérable et à haut risque d'infection à HPV et de cancer du col utérin. Selon les recommandations officielles de la Haute autorité de santé française (HAS), ces femmes immigrées bénéficient d'un frottis (HAS, 2013) ainsi que de la détection moléculaire primaire de l'ADN génital du HPV (HAS, 2019) à partir de 25-65 ans pour la détection des lésions génitales liées aux HPV. Enfin, la réponse immunitaire à IgG et les réactivités croisées vis-à-vis des types HPV des groupes $\alpha 7$ et $\alpha 9$, qui contiennent 7 des HR-HPV ciblés par le vaccin Gardasil-9®, ont été évaluées dans une série prospective de femmes immigrées de première génération, tant dans le compartiment systémique qu'au niveau génital et en accord avec la détection génitale de l'ADN de HPV, afin de dresser un profil assez proche de la réponse immunitaire acquise après une ancienne infection jugulée et au cours d'une infection persistante et de prédire l'efficacité éventuelle du vaccin prophylactique Gardasil-9® HPV dans une vaccination de rattrapage.

Résultats. Plus des deux tiers (68,6%, 35/51) des femmes de l'étude avaient une infection génitale à HPV (groupe I), ce qui correspondait à 56,8% (29/51) des femmes qui avaient une infection à HR-HPV et 11,7% (6/51) une infection à LR-HPV, tandis que 31,4% (16/51) des femmes de l'étude ne présentaient aucune infection à HPV (groupe II). La majorité (90,2%; 46/51) des femmes présentaient des réponses sériques en IgG contre au moins un type de $\alpha 7/\alpha 9$ HR-HPV [moyenne : 7,1 types de HR-HPV (extrêmes, 2 à 9) par femme]. Les réponses sériques d'IgG spécifiques du HPV étaient plus fréquemment détectées dans le groupe I que dans le groupe II [100% (35/35) contre 68,7% (11/16); $P = 0,002$]. Fait intéressant, un tiers (31,3%, 5/16) des femmes du groupe II étaient séronégatives pour tous les $\alpha 7/\alpha 9$ HR-HPV. La distribution des IgG génitales anti-HPV reflétait quelque peu celle des IgG sériques anti-HPV, mais le nombre moyen de réactivités des IgG vis-à-vis des types $\alpha 7/\alpha 9$ HR-HPV était significativement plus élevé dans le sérum que dans les sécrétions cervicovaginales (SCV) appariées (5,6 réactivités des IgG par femme dans le sérum versus 3,2 dans les SCV; $P < 0,001$). Les taux de réactivité croisée des IgG contre les types de HPV différents de l'ADN de HPV détecté par PCR étaient significativement plus élevés dans le sérum et les CVS dans le groupe I par rapport au groupe II. Enfin, la majorité (68,6%; 35/51) des femmes de l'étude présentaient des profils de réactivité des IgG sériques dirigés contre une moyenne de 6,1 types de HR-HPV ciblés par le Gardasil-9®, par femme et contre 3,7 types de HR-HPV ciblés par

le Gardasil-9® par femme dans les CVS, indiquant probablement plusieurs antécédents d'infections génitales par les types $\alpha 7/\alpha 9$ HR-HPV ciblés par le vaccin Gardasil-9®. La majorité (68,7%, 11/16) des femmes non infectées présentaient de faibles taux d'IgG contre les HR-HPV ciblés par le Gardasil-9®, à la fois dans le sérum (1,4 HR-HPV par femme) que dans les CVS (1,0 HR-HPV par femme). Un tiers (31,2%, 5/16) des femmes du groupe II n'ont montré aucune réponse IgG, à la fois génitale, mais également sérique, dirigée contre les génotypes $\alpha 7/\alpha 9$ HR-HPV.

Conclusions. Environ les deux tiers des immigrées africaines de première génération vivant en France incluses dans cette étude, présentaient une infection à HPV cervicale et des taux élevés d'IgG génitales et circulantes contre les génotypes HR-HPV des groupes $\alpha 7$ et $\alpha 9$, réagissant généralement de manière croisée, compromettant ainsi toute possibilité de vaccination de rattrapage. Néanmoins, environ un tiers des femmes ne présentaient aucun signe d'infection antérieure à HPV, ou elles ne présentaient que de faibles taux d'IgG génitales et circulantes contre les génotypes HR-HPV des groupes $\alpha 7$ et $\alpha 9$ et pouvaient donc être admissibles à la vaccination de rattrapage.

**Serum and cervicovaginal IgG immune responses against $\alpha 7$ and $\alpha 9$ HPV
in non-vaccinated women at risk for cervical cancer:
Implication for catch-up prophylactic HPV vaccination**

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Running head: HPV-specific serum and genital IgG in immigrant African women

Keywords: Human papillomavirus; Humoral immunity; B-cell; Gardasil-9^{  }; Virus-like particles; IgG; VLP-based ELISA; mucosal humoral immunity; systemic humoral immunity; Prophylactic vaccine; Catch-up HPV vaccination

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Abstract

Background. Cervical cancer associated with high risk-human papillomavirus (HR-HPV) infection is becoming the first female cancer in many sub-Saharan African countries. First-generation immigrant African women living in Europe are at-risk for cervical cancer, in a context of social vulnerability, with frequent lack of cervical cancer screening and HPV vaccination.

Objective. Our objective was to address immunologically the issue of catch-up prophylactic HPV vaccination in first-generation African immigrant women living in France.

Methods. IgG immune responses and cross-reactivities to $\alpha 7$ (HPV-18, -45 and -68) and $\alpha 9$ (HPV-16, -31, -33, -35, -52 and -58) HPV types, including 7 HR-HPV targeted by the Gardasil-9® prophylactic vaccine, were evaluated in paired serum and cervicovaginal secretions (CVS) by HPV L1-virus-like particles-based ELISA. Genital HPV were detected by multiplex real time PCR (Seegene, Seoul, South Korea).

Results. Fifty-one immigrant women (mean age, 41.7 years; 72.5% HIV-infected) were prospectively included. More than two-third (68.6%) of them carried genital HPV (group I) while 31.4% were negative (group II). The majority (90.2%) exhibited serum IgG to at least one $\alpha 7/\alpha 9$ HR-HPV. Serum HPV-specific IgG were more frequently detected in group I than group II (100% versus 68.7%; $P=0.002$). The distribution of serum and genital HPV-specific IgG was similar, but mean number of IgG reactivities to $\alpha 7/\alpha 9$ HR-HPV was higher in serum than CVS (5.6 IgG per woman in serum versus 3.2 in CVS; $P<0.001$). Rates of IgG cross-reactivities against HPV different from detected cervicovaginal HPV were higher in serum and CVS in group I than group II. Finally, the majority of groups I and II women (68.6% and 68.7%, respectively) exhibited serum or cervicovaginal IgG to Gardasil-9® HR-HPV, with higher mean rates in group I than group II (6.1 Gardasil-9® HR-HPV per woman versus 1.4; $P<0.01$). One-third (31.2%) of group II women did not show any serum and genital HPV-specific IgG.

Conclusions. Around two-third of first-generation African immigrant women living in France showed frequent ongoing genital HPV infection and high rates of circulating and genital IgG to $\alpha 7/\alpha 9$ HPV, generally cross-reacting, avoiding the possibility of catch-up vaccination. Nevertheless, about one-third of women had no evidence of previous HPV infection, or showed only low levels of genital and circulating HR-HPV-specific IgG and could therefore be eligible for catch-up vaccination.

Introduction

Human papillomavirus (HPV) infection is the most common viral sexually transmitted infection (STI) worldwide and high risk-HPV (HR-HPV) genotypes are responsible for 5.2% of all cancers worldwide and 7.7% of all cancers in developing countries [Scheurer 2005; Parkin 2006; Mboumba Bouassa 2018]. Among the thirteen HR-HPV (-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59 and -68) [Bouvard 2009], HPV-16 and HPV-18 cause about 70% of cervical cancers in women worldwide [de Sanjose 2010]. Most genital HR-HPV types cluster in the $\alpha 7$ (HPV-18, -45 and -68) and $\alpha 9$ (HPV-16, -31, -33, -35, -52 and -58) species [de Villiers 2004].

In sub-Saharan Africa, cervical cancer associated with persistent cervical HR-HPV infection is currently the first female cancer in many countries [De Vuyst 2013; Ferlay 2015]. According to the World Health Organization (WHO), cervical cancer will kill annually about half of a million women by the next decade, mostly in sub-Saharan Africa where HIV epidemic and other risk factors are worsening the burden of this cancer [Ferlay 2015; WHO 2015]. Thus, cervical cancer has become progressively one of the main public health challenges to overcome in sub-Saharan Africa [Mboumba Bouassa 2017].

One of the main strategies against cervical cancers is the primary prevention against HPV infection by the prophylactic HPV vaccination of young girls [Mboumba Bouassa 2017]. Three current virus-like particles (VLP)-based HPV vaccines for the prevention of HPV infection and related diseases have been designed and developed, such as the recently second licensed nonavalent Gardasil-9[®] vaccine from Merck & Co. Inc. (Kenilworth, NJ, USA) containing VLPs from HPV-6 and HPV-11, as well as two $\alpha 7$ (HPV-18 and HPV-45) and five $\alpha 9$ (HPV-16, -31, -33, -52 and -58) HR-HPV [Harper & DeMars 2017; Pinto 2018]. Prophylactic VLP-based HPV vaccines have been extensively evaluated in large clinical trials including populations of various origins with wide age ranges, and have demonstrated good safety and very high efficacy to confer strong and long-lasting protection against HPV infection and related diseases [Lockett & Feldman 2016; Pinto 2018; Ruiz-Sternberg 2018; St Laurent 2018; Chen 2019; de Sanjosé 2019; MacIntyre 2019; Mavundza 2019; Toh 2019; Zhu 2019]. According to the current WHO recommendations [WHO 2017], sexually active adult women more than 15 years may be eligible for catch-up HPV vaccination to complete secondary prevention of HPV-associated cervical precancerous lesions and cervical cancer using regular molecular HPV test and cervical cytology [Mboumba Bouassa 2017].

In the years 2010, African migration flows involved around 500,000 immigrants every year to Europe [Flahaux & De Haas 2016]. Around 300,000 immigrant women originating

from sub-Saharan Africa are living in France [INSEE 2015]. Most of first-generation African immigrant women arrive in Europe at the beginning of their adult life and most of them are already sexually active [INSEE 2015; Flahaux & De Haas 2016]. First-generation immigrant African women living in Europe could harbor an infectious profile reflecting the epidemiology of their country of origin where cervical HR-HPV infection is highly prevalent [Bruni 2010] and exacerbated by the so-called “syndemic” synergy played by HIV epidemic and other STIs [McCloskey 2017]. Immigrant women from sub-Saharan Africa and living in Europe show high prevalences of genital HPV infection, with at least twice more infection by HR-HPV than the European female population [Chironna 2010; Tornesello 2011]. In addition, women with an immigrant background are subjected to lower HPV vaccine initiation and completion [van der Wal 2005; Bruno 2014; Grandahl 2017; Møller 2018], and they are less screened for cervical cancer in their lifetime than women born in Europe [Grillo 2012a; Grillo 2012b]. Taken together, first-generation African immigrant adult women living in Europe, especially those infected with HIV, should be at very high risk for developing cervical cancer. Catch-up HPV vaccination among first-generation immigrant women addresses an important challenge. Indeed, the limited access of cytological or molecular cervical screening are both hampering the global success of the cervical cancer prevention in this vulnerable population [Bosch 2016; Bosch 2018; Stern & Roden 2019]. However, it is still unclear whether catch-up HPV vaccination of immigrant women who are sexually active since a while at time of vaccine introduction would be feasible and beneficial.

During primary HPV infection, the uptake of the virus into epithelial cells is thought to activate the adaptive immune system via the local antigen-presenting cells (APCs) [Roden & Stern 2018]. Thereafter, the activated APCs migrate to the local or regional lymph nodes and stimulate viral-antigen-specific CD4⁺ T cells which participate to the HPV-specific adaptive immune response in activating CD8⁺ T cells cytotoxic as well as B cells-derived humoral responses. These two arms of the immune response help to control and clear HPV infection, while in some cases, the virus can escape the host immune defenses and persist latently for many years [Roden & Stern 2018]. Natural B cells-derived humoral responses to HPV has been described in two mucosal compartments, including the cervicovaginal secretions [Wang 1996] and the saliva [Marais 2001], as well as in the systemic compartment [Gissmann 1996; Carter & Galloway 1997; Carter 2000; Touzé 2001; Stanley 2010; Scherpernisse 2013]. In the cervicovaginal compartment, HPV-specific IgA, secretory-IgA (s-IgA) and IgG, but not IgM, have been evidenced at low levels [Wang 1996; Sasagawa 2003; Mbulawa 2008]. Cervicovaginal HPV-specific monomeric IgA may be debris of genitally produced s-IgA or serum-derived transudative IgA. Interestingly, cervicovaginal

HPV-specific IgG are 2.5 times more abundant than cervicovaginal IgA and s-IgA to HPV [Hagensee 2000], as previously demonstrated for HIV infection [Belec 1995]. These findings point the critical interest to evaluate the HPV-specific immune IgG response in cervicovaginal secretions as a marker of previous or ongoing HPV infections. In the systemic compartment, both HPV-specific monomeric IgA and IgG are commonly detected [Sasagawa 1998], while low levels of HPV-specific IgM have been reported in serum in only one study [Petter 2000], but never in mucosal secretions. Circulating HPV-specific antibodies responses reflect past-infection with a 50-70% rate of seroconversion in infected women [Dillner 1999; Carter 2000] or persistent viral infection [Sasagawa 1998]. Naturally derived HPV-specific IgG antibodies from single-positive sera were highly genotype-specific, while in multi-positive sera, cross-reactive antibodies were observed both within and between some $\alpha 7$ (HPV-18 and -45) and $\alpha 9$ (HPV-16, -31, -33, -52 and -58) strains [Scherpernisse 2013]. Serum HPV-specific IgA immune responses are much more transient than anti-HPV IgG serum responses [Carter & Galloway 1997; Dillner 1999], and could have a regulatory role to the HPV-specific cellular immune response to clear the virus [Bontkes 1999]. The serological IgG immune response to HPV persists over time, it is associated with the cumulative number of lifetime sex partners [Dillner 1999], and it constitutes the “gold standard” serological test for past-history or ongoing HPV infection or reinfection [Carter & Galloway 1997; Dillner 1999].

The aim of the study was to address immunologically the issue of catch-up HPV vaccination in first-generation African immigrant women living in France, which constitute a vulnerable and high-risk population for HPV infection and cervical cancer. According to the official recommendations of the French National Authority for Health (Haute Autorité de Santé, HAS), these immigrant women benefit from 25 to 65 years of Pap smear cytology [HAS 2013] as well as primary molecular detection of genital HPV DNA [HAS 2019]. The IgG immune responses against the 7 HR-HPV contained in Gardasil-9® vaccine and cross-reactivities to $\alpha 7$ and $\alpha 9$ HPV types, were evaluated in a prospective series of first-generation immigrant women, in both systemic and genital compartment and in relationship with genital HPV DNA detection, in order to approach the most persisting immune response to HPV during past-infection or ongoing infection and to predict possible efficiency of catch-up prophylactic Gardasil-9® HPV vaccine.

Material and methods

Enrolment, selection criteria and study population. The study population consisted of African immigrant women living in France recruited for the ANRS (Agence Nationale de Recherches sur le Sida et les hépatites virales, Paris, France) ImmiPap study (Figure 1). The

ImmiPap study consists in the description of the molecular epidemiology of genital HPV infection in first-generation immigrant adult women coming from HPV endemic countries in sub-Saharan Africa, and living in France, with the ultimate aim to prevent, diagnose and cure cervical cancer. Study women benefited of the recently 2019-revised HAS recommendations for the primary molecular screening of genital HPV infection in women aged from 25 to 65 years [HAS 2019]. The ImmiPap study finally focuses on HPV prophylactic vaccination in immigrant women who are at high risk for cervical cancer.

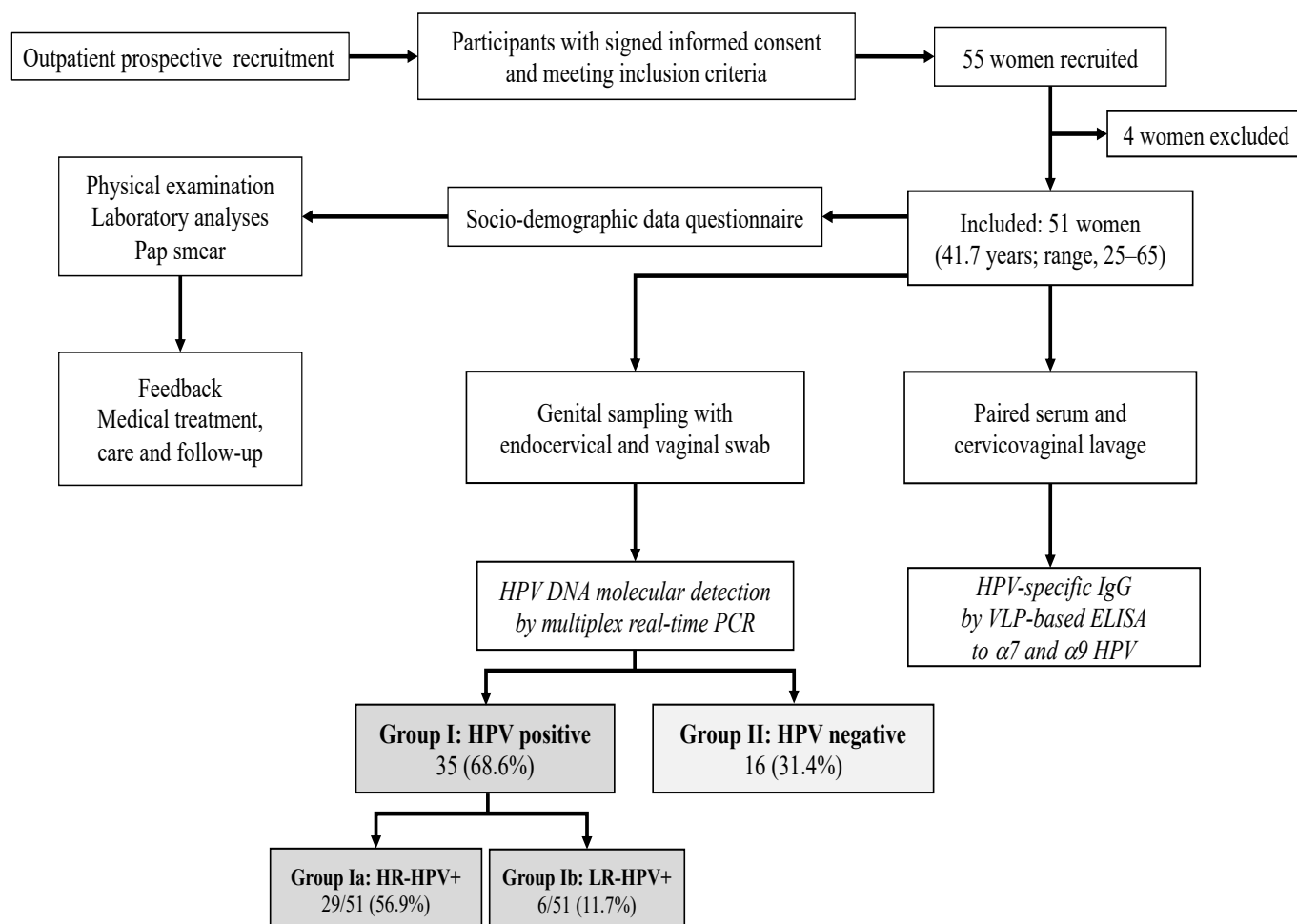


Figure 1. Flow diagram of the ImmiPap study. Three biological samples were obtained for study analyses, including a serum sample, a genital swab of endocervix and vaginal walls, and a standardized cervicovaginal lavage with 3 mL of PBS. HPV DNA detection was carried out on nucleic acid extracted from swab-collected genital secretions by multiplex Anyplex™ II HPV28 real-time PCR (Seegene, Seoul, Korea). The group I of study women showed HPV DNA in their genital secretions, including HR-HPV DNA with or without associated LR-HPV (group Ia) or exclusively LR-HPV DNA (group Ib); the group II of study women did not show detectable HPV DNA in their genital secretions. The paired serum and acellular part of cervicovaginal lavage samples were analyzed for serum and genital HPV-specific IgG, respectively, using virus-like particles-based indirect ELISA assay against HR-HPV belonging to α7 (HPV-18, -45 and -68) and α9 (HPV-16, -31, -33, -35, -52 and -58) groups.

ELISA: Enzyme-linked immunosorbent assay; HR-HPV: High risk-HPV; LR-HPV: Low risk-HPV

First-generation immigrant African women attending the Centre Gratuit d'Information, de Dépistage et de Diagnostic (CeGIDD) of the Centre Hospitalier Régional d'Orléans were prospectively included in 2019. The CeGIDD is an outpatient consultation service providing HIV, STIs, hepatitis and HPV prevention, screening and care for the general adult population.

The inclusion criteria were to be a first-generation African immigrant women according to the French National Institute of Statistics and Economic Studies [INSEE 2015], being aged between 25-65 years, being sexually active, having no genital troubles at physical examination, having signed the informed consent form, having fully completed the study questionnaire and providing the blood and genital samples required for the virological and immunological studies. Exclusion criteria included age less than 25 years and more than 65 years, not willing to participate to the study or to answer the face-to-face sociodemographic and medical data questionnaire.

After signed the informed consent form, included study women were invited to fulfill a face-to-face questionnaire that included socio-demographic characteristics and behavioral data such as age, marital status, social occupation, education level, past-history of STI, HIV status and also sexual behavioral characteristics such as the number of lifetime sexual partners, frequency of condom use and the age at first sexual intercourse. Study women were subjected to physical examination with appropriate laboratory analyses when necessary, and to a routine Pap smear cytology, as recommended by the HAS in women aged from 25 to 65 years for HPV-related genital lesions detection [HAS 2013]. They also benefited from free STIs screening, including HIV, Chlamydia trachomatis, genital herpes and syphilis. All women received an information session on HIV, STIs and cervical cancer. Women diagnosed positive for any STIs received adequate care.

Samples and processing. After completing the questionnaire, a clinician performed genital sampling of the endocervix and the vaginal walls using a flocked swab (Copan Diagnostic Inc., California, USA), frozen at -80°C . Afterwards, whole cervicovaginal secretions were collected using a standardized vaginal washing, with 3 mL of phosphate-buffered saline (PBS) (Thermo Fisher Scientific, Waltham, MA, USA), as previously described [Bélec 1995]. The samples, corresponding to an approximately 10-fold dilution the original secretions [Belec 1995], were centrifuged to remove cells, debris, and insoluble mucus, prior to be stored at -80°C . For each woman, 5 mL of venous blood were obtained in dry tube, and the serum was retrieved to be further aliquoted in 1.5 mL cryotubes before to be stored at -80°C .

Frozen genital and serum samples were transported in frozen ice packs to the virology laboratory of the hôpital européen Georges Pompidou, Paris, France, for virological and serological analyses.

Cervical cytology was carried out by cytoscreeners and pathologists blinded to the outcomes of HPV testing. Cytological results were classified according to the Bethesda system 2001 [Solomon 2002].

HPV detection and genotyping. Genital swab from each included women were subjected to DNA extraction using the DNeasy Blood and Tissue kit (Qiagen, CA, USA) and eluted in 100µL of elution buffer according to the manufacturer instructions. Genital HPV DNA was detected in eluted total DNA from vaginal swab and genotyped using the multiplex real-time PCR assay Anyplex™ II HPV28 (Seegene, Seoul, South Korea), as previously described [Mboumba Bouassa 2019].

Human papillomavirus virus-like particles production. VLPs for nine HPV genotypes (HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-45, HPV-52, HPV-58, and HPV-68) were produced using bicistronic plasmids vectors (p16sheLL, p18sheLL, p31sheLL, pVITRO-HPV33L1L2, p35sheLL, p45sheLL, p52sheLL, p58sheLL and pVITRO-HPV68L1L2) incorporating each one, a copy of the major L1 and minor L2 capsid protein encoded gene specific for each of the nine HPV genotypes, as described previously [Buck & Thompson 2007]. Plasmids p16sheLL, p18sheLL, p45sheLL, p52sheLL and p58sheLL were a gift from John Schiller (Addgene plasmid # 37320; # 37321; # 37322; # 37323; # 46950; # 37324) [Buck 2006; Roberts 2007; Kondo 2007; Kondo 2008]. While plasmid p35sheLL was a gift from Simon Beddows (Addgene plasmid # 40626) [Draper 2011] and pVITRO-HPV33L1L2 and pVITRO-HPV68L1L2 were gifted by Richard Roden (Addgene plasmid # 52493 and # 52587) [Kwak 2014]. All the plasmids were available in the plasmid repository, Addgene (www.addgene.org).

The production of VLP stocks was performed as previously described [Buck & Thompson 2007] using the papillomavirus vectors production protocol (<https://ccrod.cancer.gov/confluence/display/LCOTF/PseudovirusProduction>) edited by the Laboratory of Cellular Oncology of the Center for Cancer Research (National cancer institute, National Institute of Health, Rockville Pike, Bethesda, Maryland, USA). Briefly, 293 TT cells were transfected with each of the L1/L2 plasmid vectors and incubated during 72 hours at +37°C. Cells were then lysed in a buffer (0.5% Nonidet™ P 40 Substitute/1X PBS, Sigma-Aldrich, Saint-Louis, Missouri, USA) before being sonicated 3 times for 15 seconds cycles to

break the nuclear membrane and thereby releasing the VLPs. The obtained nuclear extracts were purified by ultracentrifugation at 30,500 rpm (SW 32 Ti Swinging-Bucket rotor, Beckman Coulter, Inc., Brea, CA, USA) for 26 hours at +4°C in a cesium chloride (ClCs) gradient. The fraction containing the VLPs was recovered and diluted in Dulbecco's phosphate-buffered saline (DPBS) (Thermo Fisher Scientific, Waltham, MA, USA) and stored at +4°C before being used for serological analyses. Anti-HPV16-L1 antibody [CamVir 1, ab69] (Abcam, Cambridge, UK) was used as quality control to ensure the specificity of VLP-16 and -31, as well as the non-cross-reactivity of the other VLPs with antibody specific for HPV-16 and phylogenetically close genotypes. Likewise, the anti-HPV18-L1 antibody [abx110595] (Abbexa Ltd, Cambridge, UK) was used as quality control for VLP-18. Finally, the anti-HSV-1/HSV-2 (herpes simplex virus type 1/2 glycoprotein B) antibody (ABIN457436, Antibodies-online, GmbH, Aachen, Germany) was used to ensure the non-cross-reactivity with other virus species.

VLP-based IgG ELISA. An “in-house” VLP-based indirect enzyme linked immunosorbent assay (ELISA) was carried out on paired serum and acellular part of vaginal lavage of each included woman. Briefly, a 96 well ELISA microtiter plates (NUNC F96 MaxiSorp™, Thermo Fisher Scientific, Waltham, MA, USA) were coated with 100µL of each purified VLP (VLP-16, VLP-18, VLP-31, VLP-33, VLP-35, VLP-45, VLP-52, VLP-58, and VLP-68) diluted at 1:10 in PBS and incubated overnight at +4°C. Each well was blocked with 200 µL of PBS containing 1% of fetal bovine serum (Dominique Dutscher SAS, Brumath, France) during 1 hour at room temperature and vigorously decanted to remove the exceeding and non-coated antigens. The paired serum and genital lavages from each woman were then diluted (1:10 and 1:100 for genital lavage and serum, respectively) in PBS and charged in the corresponding wells for an incubation period of 1 hour in the dark, at +37°C. After each incubation step, the plate was washed 4 times with PBS containing 0.1% of Tween® 20 (Sigma-Aldrich, Saint-Louis, Missouri, USA). Then, the plate was incubated at +37°C, in the dark, during 1 hour with an horseradish peroxidase (HRP)-conjugated mouse anti-human IgG monoclonal antibody [ab7499] (Abcam, Cambridge, UK) diluted in PBS (1:6000, as recommended by the manufacturer) in order to detect serum and genital IgG specific for each VLP. Following incubation and washing steps, 100 µL of enzyme substrate [hydrogen peroxide (H₂O₂) Sigma-Aldrich, Saint-Louis, Missouri, USA] and chromogen (orthophenylenediamine, Sigma-Aldrich) were added in wells and the plates were placed in the dark for a final incubation period of 30 minutes to allow color development. Reactions were stopped with 0.1 M of sulfuric acid (H₂SO₄, Thermo Fisher Scientific, Waltham, MA, USA),

and optical density (OD) was read between 450 and 620 nm on a microplate reader (ETIMAX 3000, DiaSorin, Saluggia, Italy). All samples were tested in triplicate for each VLP type.

Cutoff positivity values to define HPV-specific IgG seropositivity were calculated independently for each HPV type by analyzing the mean OD values obtained from children's sera according to the cutoff algorithm recommended by the global HPV LabNet (mean OD value of a negative control serum panel plus 3 SD) [Eklund 2012; Kelly 2018].

A panel of sera from 20 French children less than 5 years old was used as negative control for serological assays. In addition, a pool of sera from individuals having received the three doses of Gardasil-9® vaccine (Merck & Co. Inc., NJ, USA) constituted the positive control in serological assays for each of the seven HR-HPV types targeted by the Gardasil-9® vaccine (VLPs from HPV-6, -11, -16, -18, -31, -33, -45, -52 and -58).

Statistical analysis. Means and standard deviations (SD) were calculated for quantitative variables and proportions for qualitative variables. Mann-Whitney test was used to compare mean seroreactivity for each HPV type between women. Wilcoxon signed ranks test (quantitative variables) and McNemar's Chi-squared test (qualitative variables) were used to compare the mean IgG reactivities and the prevalences of each HPV-specific IgG between paired serum and cervicovaginal samples.

Ethic statement. This study was part of the ARNS-ImmiPap study and the Scientific Committee of the Centre Hospitalier Régional d'Orléans formally approved the study. All the included women gave their informed signed consent to participate to the study.

Results

Sociodemographic and clinical characteristics of study population. A total of 55 African first-generation immigrant women (mean age, 41.7 years; range, 25-65) living in France for an average of 10.7 years (range, 1-32 years) was prospectively recruited (Table 1). Four of them were excluded because of menstruations at time of enrollment. Finally, 51 women fulfilling the inclusion criteria were included. The group I of study women (68.6%, 35/51) showed HPV DNA in their genital secretions, including HR-HPV DNA associated, or not, with low risk-HPV (LR-HPV) (group Ia) or exclusively LR-HPV DNA (group Ib); the group II of study women (31.4%, 16/51) did not show detectable HPV DNA in their genital secretions (Table 1).

Table 1. Sociodemographic characteristics, HIV serostatus, past-history of sexually transmitted infections and cervical cytological results in study women, according the presence or absence of HPV DNA detected by multiplex real-time PCR in swab-collected cervicovaginal secretions.

	All study women (N=51)	Women with genital HPV DNA (group I; N=35)	Women without genital HPV DNA (group II; N=16)
Age [mean, (range); years]	41.7 (25-65)	42.8 (28-65)	39.6 (24-50)
Time of stay in France [mean, (range); years]	10.7 (1-32)	9.8 (1-32)	11.43 (1-27)
Marital status			
Single [n,(%)]	23 (45.1%)	13 (37.2%)	10 (62.5%)
In couple [n,(%)]	20 (39.3%)	15 (42.8%)	5 (31.2%)
Divorced [n,(%)]	7 (13.7%)	6 (17.2%)	1 (6.3%)
Widowed [n,(%)]	1 (1.9%)	1 (2.8%)	0 (0%)
Employment status			
Employed [n,(%)]	19 (37.2%)	11 (31.4%)	8 (50.0%)
Unemployed [n,(%)]	29 (56.8%)	22 (62.9%)	7 (47.7%)
Student [n,(%)]	3 (5.9%)	2 (5.7%)	1 (6.3%)
Sexual behavior			
Age at first sexual intercourse [mean, (range); years]	18.1 (12-25)	18.1 (12-25)	18.1 (14-22)
Number of sexual partners in the last year [mean, (range)]	1.1 (1-2)	1.1 (1-2)	1.1 (1-2)
Condom use			
Always [n,(%)]	6 (11.7%)	5 (14.3%)	1 (6.3%)
Occasionally [n,(%)]	30 (58.9%)	19 (54.3%)	11 (68.7%)
Never [n,(%)]	15 (29.4%)	11 (31.4%)	4 (25.0%)
Seropositivity for HIV infection [n,(%)]	37 (72.5%)	26 (74.3%)	11 (68.7%)
Past-history of sexually transmitted infections* [n,(%)]	3 (5.8%)	1 (2.8%)	2 (12.5%)
Cervical cytological results			
Normal cytology [n,(%)]	43 (84.4%)	28 (80.0%)	15 (93.7%)
LSIL [n,(%)]	6 (11.7%)	5 (14.3%)	1 (6.3%)
HSIL [n,(%)]	2 (3.9%)	2 (5.7%)	0 (0%)

*Sexually transmitted infections included *Chlamydia trachomatis*, herpes simplex virus-type 2 and syphilis.

HSIL: High grade squamous intraepithelial lesion; LSIL: Low grade squamous intraepithelial lesion.

The study population was mainly constituted by HIV-infected women (72.5%; 37/51) while 27.5% were HIV-negative. Single women (45.1%) were more represented than women living in couple with a male partner (39.3%). Only one-third (37.2%) of women reported to have a paid job while more than half of them (56.9%) were unemployed, including a minority (5.9%) of students. The majority of women started their sexual life at 18.1 years (range, 12-25), and all, but 3, reported having started their sexual life in their home country, longtime before moving to France. Most women reported having had an average of 1.1 sexual partners (range, 1-2) during the last 12 months. Most women used generally condom occasionally

(58.9%); nearly one-third (29.4%) of study women never used condom; and only a minority used condom (11.7%) consistently. The large majority of study women were free of STIs (94.1%), while only a minority (5.9%) reported past-history of STIs. Finally, most women showed normal cytological results (84.4%) and only 8 (15.7%) women exhibited abnormal cytology including 6 women with low-grade squamous intraepithelial lesion (LSIL), and 2 with high-grade squamous intraepithelial lesion (HSIL). Women shedding genital HPV DNA showed a trend to harbor more frequently abnormal cytology results (7 cases, 20.0%) than women without genital HPV DNA (1 case, 6.3%), but the difference was not significant. Interestingly, both cases of HSIL were observed in the group I of women carrying genital HPV DNA.

There was no significant difference between the groups I and II for sociodemographic as well as clinical findings (not shown).

No study women were vaccinated against HPV infection and all of them had never undergone cervical Pap smear cytology before the study inclusion. Furthermore, all study women reported to have never been screened for primary molecular detection of HPV DNA in their genital secretions.

HPV DNA detection and genotyping. The Table 2 summarizes the distribution of HPV genotypes in study women. More than two-third (68.6%, 35/51) of women carried genital HPV DNA (group I), including 56.8% (29/51) carrying genital HR-HPV DNA (group Ia) and a minority (11.7%, 6/51) genital LR-HPV DNA (group Ib). Nearly one-third (31.4%; 16/51) did not show by molecular biology any HPV DNA in their genital secretions (group II). Genital HPV infection profiles with multiple HPV genotypes were observed in around one-quarter of study women (23.5%), with an average of 2.1 HR-HPV (range, 1 to 4) per genital swab sample. The $\alpha 7$ HR-HPV-68 was the most frequently (19.6%) detected genotype, followed by the $\alpha 9$ HR-HPV-58 (13.7%). HR-HPV-16 and HR-HPV-18 were respectively detected in 7.8% and 9.8% of study women. Apart from the Gardasil-9® vaccine HPV-31 type detected in 7.8% of women, each of the other Gardasil-9® types (HPV-6, HPV-11, HPV-33, HPV-45 and HPV-52) were detected only in one (1.9%) woman. Except for the HPV-51 (7.8%), the other HR-HPV types not targeted by the Gardasil-9® vaccine (HPV-35, HPV-39, HPV-56 and HPV-59) were rarely observed.

* HPV genotypes detected by Anyplex HPV 28 molecular test kit (Seegene, Seoul, Korea): LR-HPV: 6; 11; 40; 42; 43; 44; 53; 54 and 70, HR-HPV: 16; 18; 31; 33; 35; 39; 45; 51; 52; 56; 58; 59 and 68, Possibly oncogenic HPV: 26; 61; 66; 69; 73 and 82;

** α 7 high risk-HPV types: HPV-18, -45 and -68; α 9 high risk-HPV types: HPV-16, -31, -33, -35, -52 and -58;

μ HPV-16, -18, -31, -33, -45, -52 and -58 are the HR-HPV genotypes targeted by the Gardasil-9® vaccine;

§ Concordance is the n/n' ratio corresponding to the number (n) of cervicovaginal HPV types detected by PCR out of the number (n') of detectable HPV-specific serum or cervicovaginal IgG positivity against the same HPV types: 1/1, 2/2 or 3/3 correspond to perfect concordance; 0/1, 0/2, 0/3 correspond to lack of concordance and 1/2 correspond to serum or cervicovaginal reactivity against 1 out of the two HPV types detected by PCR;

§ Cross-reactivity corresponds to the number of serum or cervicovaginal HPV-specific IgG reactivities against HPV types other than those detected in genital secretions of women from group I, and to the number of serum or cervicovaginal HPV-specific IgG reactivities against any HPV types in women from group II;

£ Not applicable for women infected with HPV types other than the 9 HR-HPV types (HPV-16, -18, -31, -33, -35, -45, -52, -58 and -68) used to produce the virus-like particles constituting the HPV antigens in the IgG ELISA, or for women not infected by HPV in their genital tract.

CVS: Cervicovaginal secretions; ELISA: Enzyme-linked immunosorbent assay; HPV: Human papillomavirus; HR-HPV: High risk-HPV; ID: Identification number; IgG: Immunoglobulin G; Low risk-HPV: LR-HPV; NA: Not applicable; Se: Serum

Serum HPV-specific IgG immune response. The Table 2 summarizes serum and vaginal humoral IgG immune responses against α 7 and α 9 HR-HPV types in study women.

In the systemic compartment, the large majority of women (90.2%; $n=46$) exhibited serum IgG responses against at least one α 7/ α 9 HR-HPV type, while only five women (9.8%) were seronegative for all the HPV types studied. Profiles of seropositivity to multiple HPV types were frequent (76.5%), on average against 7.1 VLP-HPV types (range, 2 to 9) per woman. Nearly half of women (43.1%) were seropositive for all the nine HPV types. The Figure 2 A depicts the α 7 and α 9 HPV-specific serum IgG responses of the study women. Overall, women were mostly seropositive for α 9 HPV types, with serum IgG against HPV-31 (80.4%) being the most prevalent HPV-specific reactivity, followed by IgG to HPV-16 (76.5%), HPV-52 (68.6%) and HPV-58 (60.8%). The serum IgG to HPV-18 were detected in 58.8% of women, followed by IgG to HPV-68 (56.8%), HPV-45 (56.8%), HPV-35 (54.9%) and HPV-33 (45.1%).

Serum HPV-specific IgG responses were more frequently detected in women with genital HPV (group I) than in women without genital HPV (group II) [group I: 100% (35/35) versus group II: 68.7% (11/16); $P=0.002$] (Figure 3). Furthermore, the mean number of serum IgG reactivities to α 7/ α 9 HR-HPV types were significantly higher in women from group I than in those from group II (group I: 7.7 HR-HPV types per woman, range: 1 to 9, versus group II: 1.0 HR-HPV type per woman, range: 0 to 2; $P<0.001$) (Figure 2 B and C). Interestingly, while all (100%) women belonging to group I were seropositive against at least one HR-HPV type, one-third (31.3%, 5/16) of women from group II were seronegative for all the HR-HPV types investigated.

Women from group II harbored serum IgG only reactive to α 9 HPV types, mostly to HPV-16 and HPV-31 (43.7%, 7/16) and less frequently to HPV-35 and HPV-52 (6.2%, 1/16) (Figure 2 C). In addition, all women from group Ia, but one (ID#26), harbored concordant

HPV-specific serum IgG immune responses for at least one of the HR-HPV types detected by PCR in their swab-collected cervicovaginal secretions (Table 2). Finally, the mean number of cross-reacting HPV-specific serum IgG reactivities against HPV types different from detected cervicovaginal HPV DNA was higher in women from group I than in those from group II (group I: 6.7 serum IgG reactivities per woman, range 1-8, versus group II: 1.0, range 0-2; $P < 0.001$) (Table 2).

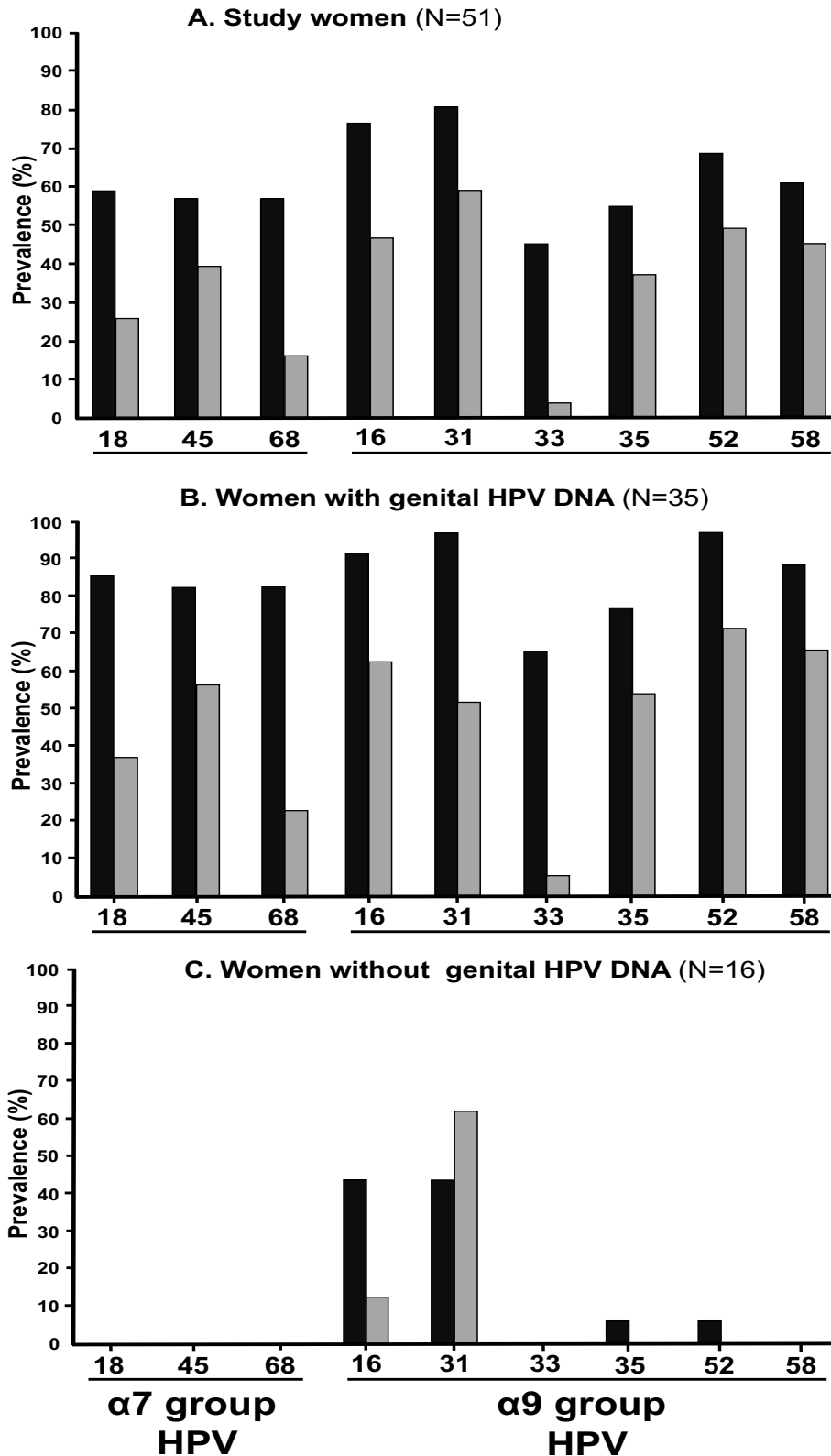


Figure 2. Prevalences of IgG reactivities to $\alpha 7/\alpha 9$ HPV types in paired serum and cervicovaginal secretions from study women with positive HPV DNA detection in their genital tract (group I; N=35) (black hatched boxes), and from women with negative cervicovaginal HPV DNA detection (group II; N=16) (white boxes). The prevalences of HPV-specific IgG reactivities by virus-like particles-based ELISA in the systemic as well as genital compartment were higher in women carrying HPV DNA than in women without detectable cervicovaginal HPV.

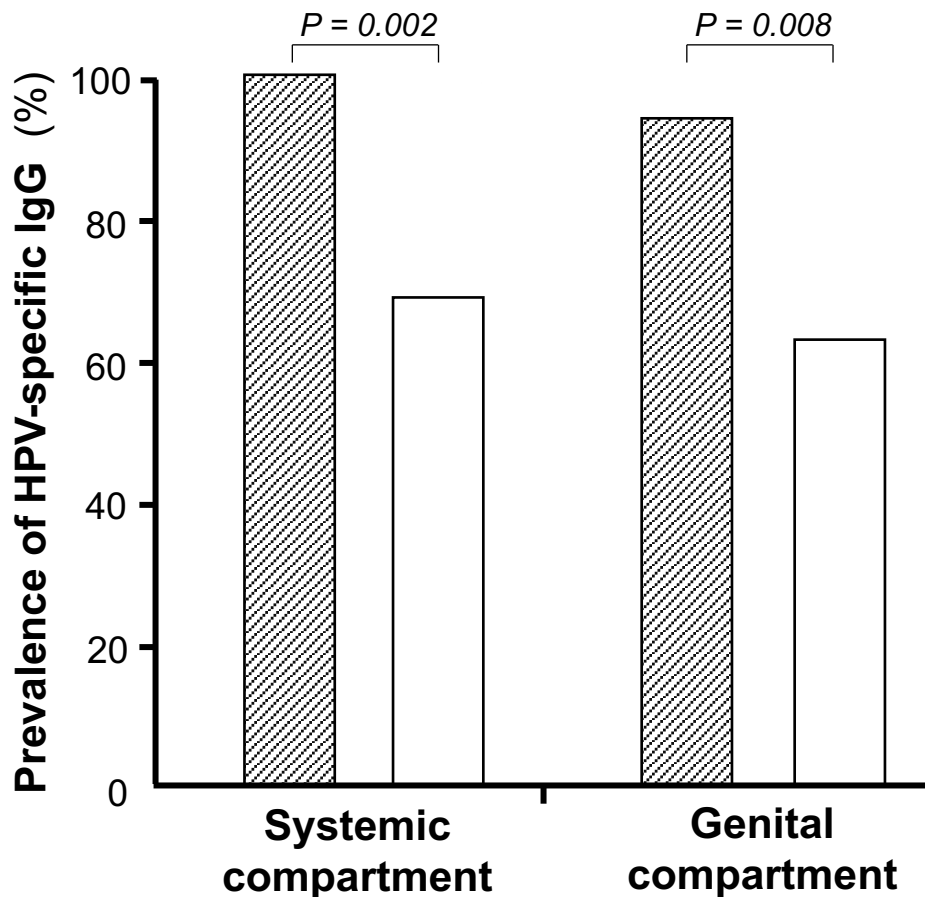


Figure 3. Prevalences of IgG reactivities to $\alpha 7/\alpha 9$ HPV types in paired serum (black boxes) and cervicovaginal secretions (grey boxes) according the presence of genital HPV DNA. A. Whole study population of first-generation immigrant African women (N=51); B. Women with positive HPV DNA detection in their genital tract (group I; N=35); C. Women with negative cervicovaginal HPV DNA detection (group II; N=16).

Cervicovaginal HPV-specific IgG immune response. In the genital compartment, more than three-quarter (84.3%) of study women showed cervicovaginal IgG against at least one of the nine $\alpha 7/\alpha 9$ VLP-HR-HPV used as antigens, while no genital HPV-specific IgG could be detected in 15.7% (8/51) of women (Table 2 and Figure 2 A). Genital IgG responses against several HR-HPV types were observed in more than half of study women (56.8%), with an average of 5.1 genital IgG reactivities to HR-HPV types (range, 2 to 9) per woman. Overall, women were mostly positive for $\alpha 9$ HPV types, with genital IgG against HPV-31 (58.8%) being the most prevalent HPV-specific reactivity, followed by IgG to HPV-52 (49.1%), HPV-16 (47.1%), and HPV-58 (45.1%). The other genital IgG were detected against HPV-45 (39.2%), HPV-35 (37.2%), HPV-18 (25.5%), HPV-68 (15.7%) and HPV-33 (3.9%).

Similarly to the systemic compartment, genital HPV-specific IgG responses were more frequently detected in women with HPV genital shedding than in those without genital

HPV [group I: 94.3% (33/35) versus group II: 62.5% (10/16); P=0.008] (Figure 3). Furthermore, the mean number of genital IgG reactivities to $\alpha 7/\alpha 9$ HR-HPV types were significantly higher in women from group I than in those from group II (group I: 4.3 HR-HPV types per woman, range: 0 to 9, versus group II: 0.7 HR-HPV type per woman, range: 0 to 2; P<0.001) (Figure 2 B and C). Interestingly, while all (100%) women belonging to group I were seropositive against at least one $\alpha 7/\alpha 9$ HR-HPV type, women from group II showed genital IgG only reactive against two $\alpha 9$ HR-HPV types (HPV-16: 12.5% and HPV-31: 62.5%) (Figure 2 B and C).

The majority of women from group I (54.3; 19/35) exhibited vaginal IgG response specific to the HR-HPV type detected in their cervicovaginal swab specimen (Table 2). However, 40.0% (14/35) of women from group I showed genital IgG response specific to other HR-HPV types than to the HPV type detected in their cervicovaginal secretions (Table 2). Finally, two (5.7%) women harboring genital DNA did not show any HPV-specific cervicovaginal IgG response.

Women from group II harbored genital IgG only reactive to $\alpha 9$ HPV types, including HPV-31 (62.5%, 10/16) and HPV-16 (12.5%, 2/16) (Figure 2 C). The mean number of cross-reacting HPV-specific genital IgG reactivities against HPV types different from detected cervicovaginal HPV DNA was higher in women from group I than in those from group II (group I: 3.8 genital IgG reactivities per woman, range 0-9, versus group II: 0.7, range 0-2; P<0.001) (Table 2).

Comparison of HPV-specific IgG immune responses in paired serum and cervicovaginal secretions. Overall, the distribution of serum HPV-specific IgG mirrored somehow that of genital HPV-specific IgG, with anti-VLP-HPV-31 (80.4% in serum and 58.8% in genital secretions), HPV-16 (76.5% in serum and 47.1% in genital secretions), HPV-52 (68.6% in serum and 49.1% in genital secretions) and HPV-58 (60.8% in serum and 45.1% in genital secretions) being the most frequently detected IgG antibodies both in the systemic and the genital compartments (Figure 2 A).

There was however numerous differences between the systemic and genital compartments concerning the prevalences of IgG reactivities, the concordances between HPV-specific IgG reactivities and the types of detected genital HPV DNA, and the cross-reactivities of HPV-specific IgG against HPV types different from genital HPV DNA, mainly in the group I of women with HPV genital shedding (Figure 2 B).

Thus, in the whole study population, the mean number of IgG reactivities to $\alpha 7/\alpha 9$ HR-HPV types was significantly higher in serum than in paired genital secretions (serum IgG:

5.6 HR-HPV types per woman, range 0 to 9, versus genital IgG: 3.2 HR-HPV types per woman, range 0 to 9; $P < 0.001$) (Table 2). These observations were more pronounced in the group I of women shedding HPV DNA (serum IgG: 7.7 HR-HPV types per woman, range 1 to 9, versus genital IgG: 4.3 HR-HPV types per woman, range 0 to 9; $P < 0.001$).

More than one-third (37.1%, 13/35) of women carrying genital HPV DNA (group I) did not show concordant IgG seroconversion profile against the ongoing HPV infection between the systemic and genital compartments (Table 2). Although these women showed serum IgG reactivities associated with HPV types detected from genital swab, they did not exhibit the same HPV-specific IgG reactivities in genital secretions. Furthermore, in the group Ia of women with genital HR-HPV DNA shedding, the concordance between HPV types detected by PCR and HPV-specific serum or cervicovaginal IgG reactivities appeared higher in the systemic than in the genital compartments. Indeed, while 26 out of the 27 (96.3%) women from the group Ia showed concordance between $\alpha 7/\alpha 9$ HR-HPV DNA and serum IgG to the same HR-HPV, only less than half of them (48.1%; 13/27) exhibited concordance between $\alpha 7/\alpha 9$ HR-HPV DNA, serum and genital IgG to the same HR-HPV ($P = 0.005$) (Table 2). Furthermore, a minority of women (7.4%; 2/27) with multiple genital HR-HPV DNA showed serum IgG responses against all the HR-HPV types detected in their genital swab, but their genital IgG responses were directed only against one of the HPV types detected by PCR (Table 2).

When excluding the IgG reactivities associated with HPV types detected from genital swab, the mean number of IgG cross-reactivities against HPV types different from genital HPV DNA was higher in serum than in paired genital secretions (serum IgG, 4.9 HR-HPV types per woman, range 0 to 8, versus genital IgG: 2.8 HR-HPV types per woman, range 0 to 8; $P < 0.001$) (Table 2).

In contrast to group I, in women without genital HPV DNA shedding (group II), anti-VLP-HPV-31 was mostly detected in cervicovaginal secretions (62.5%) than in paired serum (43.7%) (Figure 2 C). Nevertheless, in group II, the mean number of IgG cross-reactivities against HPV types different from genital HPV DNA was similar in serum and in paired genital secretions (serum IgG, 1.0 HR-HPV types per woman, range 0 to 2 versus genital IgG: 0.7 HR-HPV types per woman, range 0 to 2; $P > 0.05$) (Table 2).

HIV-infected and HIV-seronegative women harbored high rates of HR-HPV specific IgG positivity, in both serum (89.2% versus 92.8%) and genital secretions (86.5% versus 78.6%), without any significant difference ($P > 0.05$).

Finally, when considering both systemic and cervicovaginal compartments, 31.2% (5/16) of women without detectable genital HPV DNA (group II) did not show any serum and genital HPV-specific IgG immune responses to $\alpha 7/\alpha 9$ VLP-HR-HPV.

Presumed predictive efficiency of catch-up prophylactic Gardasil-9® HPV vaccine. None of study women had received HPV prophylactic vaccination, which raises the issue of possible catch-up vaccination for them.

Study women could be divided in 3 distinct categories.

The first category comprises the majority of study women (68.6%; 35/51) with genital $\alpha 7/\alpha 9$ -HR-HPV (group Ia) or other LR-HPV genotypes (group Ib). All these women harbored serum IgG reactivity profiles against an average of 6.1 Gardasil-9® HR-HPV types per woman (range, 1 to 7), and genital IgG reactivity profiles against an average of 3.7 Gardasil-9® HR-HPV types per woman (range, 0 to 7), likely indicating several past-history of genital infections by $\alpha 7/\alpha 9$ HR-HPV. Simultaneous IgG seropositivity to all HR-HPV types included in the nonavalent Gardasil-9® vaccine was observed in 68.6% (24/35) of serum or genital secretions from women belonging to group I (Table 3). However, partial simultaneous IgG seropositivity for 4 to 6 HR-HPV types included in the nonavalent Gardasil-9® vaccine could be observed in 9 of 35 (25.7%) women, as depicted in the Table 3. Thus, the majority (94.3%; 33/35) of this first category of women showed serum or cervicovaginal IgG positivities against all, or at least 4, of the seven HR-HPV types targeted by the Gardasil-9® vaccine, and could be a priori poorly eligible for catch-up HPV vaccination.

Table 3 (Annexe 4). Number of serum or cervicovaginal IgG seropositivities to $\alpha 7/\alpha 9$ HR-HPV types included in the nonavalent Gardasil-9® vaccine among study women with positive HPV DNA detection in their cervicovaginal secretions (group I; N=35), including women with HR-HPV DNA (group Ia; N=29) and women with LR-HPV DNA (group Ib; N=6), and among women with negative HPV DNA detection in their cervicovaginal secretions (group II; N=16).

The second category was the majority (68.7%, 11/16) of women from group II exhibiting serum IgG reactivity profiles against an average of 1.4 Gardasil-9® HR-HPV type per woman (range: 0 to 2) and genital IgG reactivity profiles against an average of 1.0 Gardasil-9® HR-HPV type per woman (range: 0 to 2) (Table 2 and Figure 2 C). Simultaneous IgG seropositivity for 3 to 7 HR-HPV types included in the Gardasil-9® vaccine could not be observed in these women (Table 3). Such serum or genital HPV-specific IgG profiles could be a priori eligible for possible catch-up HPV vaccination.

Finally, the third category consisted in one-third (31.2%, 5/16) of study women without detectable genital HPV DNA (group II) who, furthermore, did not show any serum and genital HPV-specific IgG immune responses to all $\alpha 7/\alpha 9$ VLP-HR-HPV (Table 2 and Figure 2 C). These women could be likely fully eligible for possible catch-up prophylactic HPV vaccination.

Discussion

We herein addressed immunologically the issue of catch-up prophylactic HPV vaccination in a consecutive series of first-generation immigrant African women living in France. Included women were frequently infected by HIV. Furthermore, no study women had been vaccinated against HPV infection and none of them had ever undergone cervical Pap smear cytology before inclusion, confirming the vulnerability of immigrant African women regarding the risk of cervical cancer. Molecular detection of HPV DNA revealed unsuspected high burden of genital shedding of HPV (68.6%) and $\alpha 7/\alpha 9$ HR-HPV (56.8%), with the non-vaccine $\alpha 7$ -HR-HPV-68, a genotype rarely found within the French female population, being the most frequently detected. These observations point the remarkable and atypical epidemiological profile of cervical HPV infection in study population. In addition, serum and cervicovaginal IgG immune responses to at least one $\alpha 7/\alpha 9$ HR-HPV type could be detected by VLP-based ELISA in the majority of included women, indicating high frequency of past- or ongoing genital HPV infection. When considering the results of molecular HPV detection in genital secretions and those of IgG immune responses to $\alpha 7/\alpha 9$ HPV types in the systemic and genital compartments, three distinct categories of women could be observed. Firstly, around two-third of women harboring genital HPV shedding (group I) showed the remarkable association of the highest prevalences of both serum and cervicovaginal IgG immune responses, the highest mean number per woman of both serum and cervicovaginal IgG reactivities to $\alpha 7/\alpha 9$ HPV types and IgG reactivities to Gardasil-9® HR-HPV types as well as the highest mean number per woman of both serum and cervicovaginal IgG cross-reactivities to $\alpha 7/\alpha 9$ HPV types different that those detected in genital secretions. In this category, concordant and cross-reacting IgG immune responses against $\alpha 7/\alpha 9$ HPV types and Gardasil-9® HR-HPV types were highly prevalent in both the systemic and genital compartments. These results are likely evidence of significant past or ongoing exposure to genital in these women and point that the reinforcement of the HPV-specific immune response by catch-up HPV vaccination in this category of women already naturally immunized against Gardasil-9® HR-HPV types will be hazardous. Secondly, around one-fifth of study women without genital HPV DNA (group II), and showed limited IgG immune response only against $\alpha 9$ HR-HPV

types targeted by the Gardasil-9® vaccine (HPV-16, -31 and -52) in the systemic or genital compartments. These findings suggest limited past exposure to genital HPV, making likely the possibility of reinforcement of the HPV-specific immune response by catch-up prophylactic HPV vaccination. Thirdly, around one-tenth of study women were not shedding genital HPV DNA (group II), and did not show any IgG immune response to $\alpha 7/\alpha 9$ HPV types and Gardasil-9® HR-HPV types, indicating a lack or only limited past exposure to genital HR-HPV. These features suggest, at least immunologically, the possibility to easily induce in this latter category of African immigrant a protective HPV-specific immune response by catch-up prophylactic HPV vaccination. Taken together, molecular detection of genital HPV DNA and assessment by VLP-based ELISA of IgG to $\alpha 7/\alpha 9$ HR-HPV types in both systemic and genital compartments from first-generation immigrant African women living in France revealed in two-third of women the unexpected high burden of past or ongoing genital infection by frequent atypical HR-HPV types, likely reflecting the primary HPV epidemiology of contamination, and a strong and cross-reacting HPV-specific IgG immune response. However, around one-third of study immigrant African women could, nevertheless, be eligible to catch-up prophylactic HPV vaccination which could be beneficial regardless of their age, as they are still sexually exposed to the risk of contamination with oncogenic HPV.

In these African immigrant women, the prevalence of cervical HPV (68.6%), frequently associated with HR-HPV genotypes (56.8%), was particularly high. Such high prevalences of cervical HPV and HR-HPV in African immigrants appear uncommon and higher than HPV prevalences previously reported in the French female general population with cervical HR-HPV infection rates oscillating from 8.0% to 22.8% in HIV-negative adult women with normal cytology [Bruni 2010; Heard 2013a; Bruni 2018] and 26.4% in HIV-positive women [Heard 2015]. A large population-based survey on HIV-positive women, including six of the main European capital cities, reported cervical HR-HPV prevalences around 35.0% [Heard 2013b], much lower than that found in our study population. On the other hand, the high prevalences of HR-HPV infection found in the present study mirror the burden of cervical HR-HPV infection commonly reported in Sub-Saharan Africa. Indeed, quite similar to our study, cervical HR-HPV prevalences in sub-Saharan Africa can reach 46.2% in HIV-negative adult women (more than 25 years) and 79.1% in HIV-positive women [Ogembo 2015; Ginindza 2017; Kelly 2017]. These observations highlight that immigrant women originating from sub-Saharan Africa and living in France, irrespectively of their HIV-status, are at least twice more infected by HR-HPV than both the French and European female population. As a consequence, adult first-generation African immigrant women living in

France, especially those infected with HIV, should be at very high-risk for developing cervical cancer.

Natural infection by HPV induce both local and systemic humoral immune response directed to HPV antigens testifying to the generalization of the humoral immune response beyond the site of virus intrusion [Beachler 2016].

In the present study, circulating IgG immune reactivities to at least one $\alpha 7/\alpha 9$ HR-HPV type were detected by VLP-based ELISA in the majority (>90%) of women, independently of HIV infection. Furthermore, the prevalences of serum HPV-specific IgG were higher in women with genital HPV shedding than in those without genital HPV, with a close concordance observed between the detected genital $\alpha 7/\alpha 9$ HR-HPV DNA and serum IgG reactivities against the same $\alpha 7/\alpha 9$ HR-HPV type. The high seroprevalence rates of IgG to $\alpha 7/\alpha 9$ HR-HPV in study women are likely the witness of both past and ongoing genital exposition to HR-HPV in these African immigrant women living in France. Indeed, circulating HPV-specific IgG may persist longtime after the natural clearance of HPV infection by the host immune system and could therefore constitute a marker of past exposition to the virus [Dillner 1999; Carter 2000; Beachler 2016; Kelly 2018]. Furthermore, the concordance between serum IgG reactivity to the VLP from the same HR-HPV type infecting the genital mucosa epithelial cells indicates activation of the humoral IgG immune response against the current genital HPV infection [Sasagawa 1998; Beachler 2016; Kelly 2018]. High prevalences of IgG seropositivity to multiple vaccine and non-vaccine HR-HPV types were previously reported to be very common in HIV-positive and HIV-negative women living in Kenya [Mbwana 2007] and Uganda [Namujju 2011] and in HIV-infected women living in South Africa [Kelly 2018]. The relatively advanced age of our study women could have also been associated with particularly high rates of HPV-specific IgG seropositivity, as reported in previous studies showing increasing HPV-specific seropositivity with older age [Castro 2014; de Araujo-Souza 2014]. Thus, the high level of circulating IgG to $\alpha 7/\alpha 9$ HR-HPV types in study population could mirror the prolonged maintenance of HPV exposure and subsequent reinforcement of the virus IgG immune response. Such high level of IgG responses likely indicate a truly increased cumulative incidence of HR-HPV infections both in HIV-negative and HIV-positive study women, and the lack of waning of HPV-specific antibodies contrarily to that generally occurred with age and the frequent decline of HPV exposure [Wang 2003].

Similarly to circulating HPV-specific IgG, cervicovaginal IgG immune reactivities to at least one $\alpha 7/\alpha 9$ HR-HPV type were detected in the majority (>84%) of enrolled women, independently of HIV infection, and most particularly in women shedding HPV DNA. The

distribution profiles of genital HPV-specific IgG mirrored somehow that of serum HPV-specific IgG. However, the mean number of IgG reactivities to $\alpha 7/\alpha 9$ HR-HPV types as well as the concordance between HPV types detected by PCR and HPV-specific serum or cervicovaginal IgG reactivities were higher in the systemic than in the genital compartments. Furthermore, less than half of women carrying genital HR-HPV DNA exhibited concordant serum and genital IgG responses against the $\alpha 7/\alpha 9$ HPV types detected in their genital secretions. Taken together, both systemic and genital humoral immune responses could not be considered as equivalent regarding the HPV-specific IgG immune response. Cervicovaginal HPV-specific monomeric IgG are thought to be mainly transudative from circulating IgG, while the possibility of an active mucosal cervicovaginal secretion of HPV-specific IgG may be induced by HPV particles uptake from the genital mucosal and in fine by the development of cervical lesions [Bontkes 1999; Tjong 2001; Sasagawa 2003; Passemore 2007]. In study women, HPV-specific genital IgG correlated only moderately with type-specific serum IgG responses, supporting the hypothesis that the genital mucosa is a poor inductor site for the immune response, with low rate of genital seroconversion and disappearance of genital IgG after the clearance of HPV infection [Bontkes 1999; Bierl 2005]. In addition, other HPV infection sites could have elicited a systemic IgG immune response to HPV. Thus, because the rectal mucosa is a powerful inductor of the systemic IgG humoral immune response [Mestecky 2005; Stanley, 2009], anal infection with $\alpha 7/\alpha 9$ HR-HPV could provide serum IgG against several $\alpha 7/\alpha 9$ HR-HPV types, without detection of any $\alpha 7/\alpha 9$ HR-HPV DNA in swab-collected genital secretions.

The proportion of women who were seropositive for multiple HPV types was much higher than the prevalence of genital multiple HPV DNA detection at inclusion, suggesting prior exposure to these types. HPV-specific IgG cross-reactivities in both systemic and genital compartments could inform on the past-history of these previous HPV infections rather than current infection. Thus, when excluding serum IgG to the HPV types detected by PCR in their genital secretions, group I women with HPV genital shedding harbored serum IgG cross-reactivity profiles against an average of 6.7 other HR-HPV types. Furthermore, the level of cross-reacting HPV-specific serum IgG reactivities was higher in women from group I than in those from group II. These findings likely indicate the occurrence of several past-history of genital infections by $\alpha 7/\alpha 9$ HR-HPV, mainly in women shedding HPV DNA at inclusion (group I), with successful control and clearance of previous infections, probably in relationship with the accumulation of sex partners during life [Dillner 1999; Carter 2000; Beachler 2016; Kelly 2018]. Considering the facts that the development of HPV-induced seroconversion is not systematic after natural infection [Carter 2000], that it often takes

several months before seroconversion can be detected [Carter 2000], and that most transient HPV infection do not elicit a detectable antibody response [Carter 2000], Dillner and colleagues suggested that HPV-specific antibodies do not play an important role in the protection or clearance of HPV infections but only represent a serological scar or at most a marker of exposure [Dillner 2007]. However, although still debated [Kelly 2018], more recent studies and meta-analysis have highlighted some evidences about the modest protecting role against subsequent infection played by the naturally acquired HPV-type specific antibodies [Malik 2009; Safaeian 2010; Castellsagué 2014; Beachler 2016; Safaeian 2018]. Note that in our series, serum or cervicovaginal antibodies to $\alpha 7/\alpha 9$ HR-HPV did not have protected women against an ongoing genital infection with phylogenetically closed genotypes of $\alpha 7$ or $\alpha 9$ HR-HPV species, likely indicating that partial protection conferred by natural derived HPV-specific is basically HPV-type specific, as previously shown in unvaccinated, HPV-antibody-positive individuals [hildesheim et al., 2007; Palmroth 2010; Scherpenisse 2013].

The immunological basis of prophylactic HPV vaccination should take into account the existing serum or cervicovaginal antibodies to $\alpha 7/\alpha 9$ HR-HPV types targeted by Gardasil-9® vaccine. The working hypothesis is that the presence of naturally derived IgG serum or cervicovaginal antibodies to $\alpha 7/\alpha 9$ HR-HPV types targeted by the Gardasil-9® vaccine could partially protect against the same type of HPV, as previously reported [Beachler 2016].

If one take into consideration that natural IgG serum or cervicovaginal antibodies to $\alpha 7/\alpha 9$ HR-HPV types protect only partially from reinfection, and cannot protect against HR-HPV phylogenetically closed to the same $\alpha 7$ or $\alpha 9$ group, the possibility exists that catch-up HPV vaccination could be interesting in this high-risk population whatever the level and cross-reactivity of its HPV-specific humoral immune responses in the systemic or genital compartments. Indeed, the HPV-specific antibody levels after vaccination in serum are 10-100 times higher as compared to naturally derived antibody levels, and the vaccine-derived specific antibodies show a significant 3 times higher avidity for VLP-HR-HPV than naturally acquired antibodies [Roteli-Martins 2012; Scherpenisse 2013]. Furthermore, the significant difference in the avidity between naturally and vaccine induced HPV antibodies may be one of the main reasons which could explain the different level of protection elicited by natural HPV infection and vaccination [Scherpenisse 2013]. Thus, prophylactic HPV vaccination could both reinforce HPV-specific humoral immunity of existing circulating or cervicovaginal antibodies to vaccine antigens and also induce new humoral immune response against Gardasil-9® $\alpha 7/\alpha 9$ HR-HPV types not yet encountered. Vaccine-induced humoral immune response against the seven $\alpha 7/\alpha 9$ HR-HPV types by catch-up multivalent HPV vaccination

could be efficient against a wide spectrum of vaccine and non-vaccine oncogenic HR-HPV types, because of high neutralizing capabilities and potent cross-reactivities within the same $\alpha 7$ or $\alpha 9$ HR-HPV species conferred by vaccine-derived HPV-specific antibodies [Draper 2011; Malagon 2012; Wheeler 2012; De Vincenzo 2013; Scherpenisse 2013; Barzon 2014; Bisset 2014]. On the other hand, the risk of possible side-effect of over HPV vaccination or hyperimmunization in previously HR-HPV-infected women remains controversial [Barbaud 2013]. Of course, all these explanations are speculative and would require further immunological corroboration.

Otherwise, current prophylactic HPV vaccines are thought to confer better protection against HPV infection mainly in individuals who have not been yet infected [Harper & DeMars, 2017; Pinto 2018]. Thus, one can consider eligible for catch-up HPV vaccination, at least theoretically, all women who have not been infected by all seven Gardasil-9® vaccine $\alpha 7/\alpha 9$ HR-HPV types or yet infected by only a minority (1 to 3) of them. In the present series, a prophylactic multivalent HPV vaccine would be beneficial depending on the group of study women. Given that 96.5% (28/29) women showed serum or cervicovaginal IgG seropositivity against 4 to 7 HR-HPV types included in the Gardasil-9® vaccine in group Ia, 16.7% (5/6) in group Ib, and 0% (0/16) in group II, catch-up HPV vaccination could be then potentially beneficial in around one-third (35.3%; 18/51) of study women who have not yet be infected by Gardasil-9® HR-HPV types or infected by only 1 to 6 of them. Catch-up HPV vaccination in these women previously infected by limited number of vaccine $\alpha 7/\alpha 9$ HR-HPV types could strengthen the already existing HPV-specific humoral immune response and induce new IgG against the other not yet encountered HR-HPV and LR-HPV types targeted by the multivalent vaccine [Olsson 2009; Castellsagué 2011].

Taken together, catch-up HPV vaccination could be poorly feasible in the category of study women shedding HR-HPV types because of marked circulating or genital IgG to vaccine $\alpha 7/\alpha 9$ HR-HPV types, likely possible in the category of women without genital HPV DNA and with weak serum or cervicovaginal IgG to vaccine $\alpha 7/\alpha 9$ HR-HPV types, and fully efficient in the category of women without genital HPV DNA and without detectable serum and cervicovaginal IgG to $\alpha 7/\alpha 9$ HR-HPV types. Note that catch-up vaccination could be proposed even in HIV-infected women, in whom better immunogenicity is seen when HIV viral replication is controlled, and when there is no overt immunodeficiency [Faust 2016; Lacey 2019].

For the majority of study women, especially those currently infected with HR-HPV, catch-up prophylactic HPV vaccination seems to be compromised. For this category of women at risk for cervical cancer, regular HPV molecular testing, cytological screening for

precancerous cervical lesions and adequate treatment remain the best suitable preventive approach to avoid the occurrence of cervical cancer [WHO 2009; WHO 2013; HAS 2013; HAS 2019].

Around one-third of study women could be eligible for catch-up prophylactic HPV vaccination. While current recommendations guidelines for HPV vaccination are primarily targeting very young adolescent girls aged 9 years up to 14 years old [WHO 2014; WHO 2017; HAS 2019; Meites 2019], prior to becoming sexually active, catch-up HPV vaccination is also recommended in females more than 15 years by the WHO [WHO 2017], before 19 years in France [WHO 2017; HAS 2019] or through age 26 years by the Advisory Committee on Immunization Practices (ACIP) in the United-States [Meites 2019], challenging somehow the initially restricted policies for HPV vaccination [Wheeler 2009; Couto 2014; Einstein 2014; Skinner 2014; Schiller 2016; Wheeler 2016]. Indeed, prophylactic HPV vaccines have shown to provide almost 67% to 90% protection against incidence and persistence of HPV infection as well as the occurrence of high-grade precancerous lesions, over 7 years after vaccination, in women older than 25 years [Wheeler 2009; Einstein 2014; Skinner 2014; Wheeler 2016], even in women with serological evidence of prior HPV exposition [Olsson 2009; Castellsagué 2011]. In October 2018, the Food and Drug Administration in USA expanded the approved age range for nonavalent Gardasil-9® vaccine use from 9 through 26 years to 9 through 45 years in women and men [FDA 2018]. In addition, ongoing clinical trials such as HPV-FASTER study already suggest that extending routine vaccination strategies to women up to age 30 years in combination with greater access to primary molecular HPV screening can hasten impact on cancer incidence [Bosch 2016; Bosch 2018; Stern & Roden 2019]. Finally, in June 2019, after reviewing evidence related to HPV vaccination of adults, the ACIP updated recommendations for catch-up vaccination and for vaccination of adults older than the recommended catch-up age, and has recommended shared clinical decision-making regarding potential HPV vaccination for people aged 27 through 45 years who could be eligible for HPV vaccination catch-up according to their immune-virological parameters [Levy & Downs 2019; Meites 2019]. Our observations bring novel and relevant immunological and virological evidences supporting the predictive efficacy of the implementation of catch-up HPV vaccination programs extended to adult women, particularly those at high risk for HR-HPV infection and cervical cancer, such as first-generation immigrant adult African women living in France. Interestingly, regardless the age criterion, primary molecular HPV testing could be used for screening of current HPV infection, as recommended by WHO [WHO 2013; Mboumba Bouassa 2017], as well as for taking decision on possible indication of catch-up HPV vaccination, as it was the case in our series.

Our study findings should be interpreted in light of some limitations. Thus, the study was constrained by limited sample size of enrolled women, in only one center, and the cross-sectional analysis. Furthermore, it should have been relevant to evaluate also the HPV-specific IgA immune response, since the natural and vaccine responses to HPV involve both IgA and IgG isotypes. Indeed, cervicovaginal HPV-specific s-IgA are only locally produced [Wang 1996], are thought to reflect current genital HPV infection [Sasagawa 2003] or HPV-related cervical lesions [Hagensee 2000], thus may reflect the acquired mucosal humoral immunity to HPV of the vaginal cavity more accurately than HPV-specific transudative IgG. This study has also important strengths, including prospective recruitment and the evaluation of the humoral immune response in two body compartments, the systemic and genital compartments, which are not equivalent regarding HPV infection. It is the first study to evaluate the immunological basis of catch-up prophylactic HPV vaccination in the vulnerable population of African immigrants.

In conclusion, this study shows several unexpected immunological combinations of systemic and genital natural HPV-specific IgG immune responses and HPV genital shedding in first-generation immigrant African women living in France. The high HPV prevalence and the limited evidence that naturally acquired antibodies protect against new DNA detection, suggest that some first-generation immigrant African women could benefit from highly multivalent HPV vaccination. Around two-third of study women showed frequent ongoing cervical HPV infection and high rates of circulating and genital IgG against $\alpha 7/\alpha 9$ HPV, generally cross-reacting, avoiding, in practice, further possibility of catch-up vaccination. Nevertheless, about one-third of women had no evidence of previous HPV infection, or showed only low levels of genital and circulating IgG against $\alpha 7/\alpha 9$ HPV and could therefore be eligible for catch-up vaccination. On a public health point of view, these observations rise the issue of possible interest to design new strategy of cervical cancer prevention, mostly in the direction of adult women at risk for HPV infection and associated lesions and who do not fit within the current WHO or national prophylactic HPV vaccine recommendations of prophylactic HPV vaccination at best prior the onset of sexual life. Instead of the age criterion, new strategy for catch-up HPV vaccination could include the results of the currently recommended primary molecular HPV testing in women aged from 30 to 65 years, and to a lesser extent assessment of the natural acquired humoral immunity to HPV (if serological tests for HPV infection will be available in routine). Studies, including mathematical modeling, assessing the efficacy and cost-effectiveness of catch-up multivalent HPV vaccine, are warranted in this population.

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Discussion

Conclusions et perspectives

A. Prévalence et distribution des principaux génotypes de HR-HPV au sein de populations africaines à risque pour l'infection à HPV

Le Tchad est l'un des pays les plus peuplés d'Afrique Centrale, avec environ 15 millions d'habitants, dont 3 millions sont des femmes de plus de 25 ans (INSEED 2014) et dans lequel aucun progrès significatif dans la prévention du cancer du col de l'utérus n'a été réalisé jusqu'à maintenant. C'est dans ce contexte que nous avons pu réaliser une étude épidémiologique (protocole GYNAUTO) permettant d'évaluer la situation de l'infection à HPV au sein d'une population à risque de femmes adultes (âge moyen : 35 ans), non-vaccinées, n'ayant jamais subi d'examen cytologique cervical et vivant à N'Ndjamena, la capitale du Tchad (Mboumba Bouassa et al., 2019). Cette étude a également permis d'évaluer un prototype d'auto-prélèvement de sécrétions cervicovaginales pour la réalisation du dépistage moléculaire de l'infection à HPV destiné aux femmes ayant un accès limité aux structures de santé spécialisée. Nous avons ainsi pu décrire pour la première fois l'épidémiologie moléculaire de l'infection à HPV au Tchad, en montrant qu'un cinquième (22,9%) des femmes incluses dans l'étude présentait une infection génitale à HPV, dont 68,9% étaient causées par des génotypes de HPV à haut-risque. De telles prévalences d'infections génitales à HR-HPV semblent être relativement plus élevées que celles couramment décrites chez des femmes adultes africaines (> 25 ans) n'ayant jamais été dépistées pour l'infection à HPV, pour lesquelles, les taux d'infection à HR-HPV n'excèdent que rarement les 50% des femmes positives à un test moléculaire. En effet, en Afrique subsaharienne, le portage génital en HR-HPV oscille généralement entre 5,4% à Djibouti (Petrelli et al., 2016), 10% à 36,5% au Nigéria (Kennedy et al., 2016; Okunade et al., 2017), 12,5% en République Démocratique du Congo (Sangwa-Lugoma et al., 2011), 18,5% à 34% au Cameroun (Catarino et al., 2016a; Kunckler et al., 2017), 19,3% au Malawi (Cubie et al., 2016), 20,3% en Tanzanie (Dartell et al., 2013), 22,2% au Rwanda (Ngabo et al., 2016), 25% en Afrique du Sud (Mbatha et al., 2017), 25,4% à 38,3% au Burkina Faso (Traore et al., 2016a; Traore et al., 2016b), 39,3% à Madagascar (Catarino et al., 2016b) et finalement 46,2% au Swaziland (Ginindza et al., 2017). Cependant, d'autres études menées également chez des femmes adultes vivant en Afrique subsaharienne ont de même signalé des prévalences élevées d'infection cervicale à HR-HPV, similaires à celles rapportées dans la présente série, avec des prévalences en HPV oncogènes variant de 60,4% au Nigéria (Akarolo-Anthony et al., 2014) à 67,9% et 68,5% en Afrique du Sud (Mbulawa et al., 2015; Mbulawa et al., 2018). Seules les études conduites chez des femmes africaines plus jeunes, mais sexuellement actives et âgées de moins de 25 ans, ont rapporté

des prévalences d'infection à HR-HPV plus élevées (70% à 84%) (Adler et al., 2013; Ebrahim et al., 2013; Watson-Jones et al., 2013; Edna Omar et al., 2017) que celles observées dans cette série de femmes adultes tchadiennes.

En ce qui concerne la distribution des génotypes de HPV, les femmes porteuses d'une infection à HR-HPV présentaient des taux remarquablement élevés (70%) d'infections causées par des génotypes couverts par le vaccin Gardasil-9®, le génotype à haut risque HPV-58 étant le génotype majoritairement détecté. De plus, cinq autres génotypes à haut-risque inclus dans le vaccin Gardasil-9® (HPV-31, HPV-16, HPV-45, HPV-52 et HPV-18) faisaient également partie des génotypes les plus fréquemment détectés. La plupart des études antérieures menées dans des pays d'Afrique subsaharienne montrent une grande hétérogénéité dans la distribution des principaux HR-HPV chez les femmes (Sangwa-Lugoma et al., 2011; Dartell et al., 2013; Akarolo-Anthony et al., 2014; Manga et al., 2015; Mbulawa et al., 2015; Ogembo et al., 2015; Catarino et al., 2016a; Catarino et al., 2016b; Kennedy et al., 2016; Ngabo et al., 2016; Petrelli et al., 2016; Traore et al., 2016a; Traore et al., 2016b; Cubie et al., 2017; Edna Omar et al., 2017; Ginindza et al., 2017; Kuncler et al., 2017; Mbatha et al., 2017; Mbulawa et al., 2018). La prédominance du génotype HPV-58 chez les femmes tchadiennes n'ayant jamais subi de dépistage cervical n'a jamais été rapportée dans d'autres cohortes de femmes d'Afrique subsaharienne et pourrait constituer une particularité de l'épidémiologie moléculaire de l'infection à HR-HPV chez les femmes vivant au Tchad. Tout comme pour le génotype HPV-58, nos observations démontrent, de façon globale, la prépondérance d'infections génitales causées par des génotypes couverts par le vaccin Gardasil-9® chez les femmes asymptomatiques vivant au Tchad, comme cela est couramment observé chez les femmes présentant une cytologie normale et celles présentant des lésions intraépithéliales de haut grade dans d'autres pays d'Afrique subsaharienne (Ogembo et al., 2015). Ces indications sont d'une importance capitale car elles permettent d'évaluer l'efficacité prédictive de la vaccination contre les principaux génotypes circulant dans la population féminine tchadienne. En effet, bien que ces femmes adultes ne semblent plus être éligibles pour la vaccination selon les recommandations de l'OMS (âge de la vaccination : 9 à 14 ans, avec rattrapage jusqu'à 19 ans) (WHO 2017), le profil de distribution des principaux génotypes détectés chez ces femmes nous renseigne sur les génotypes circulant dans la population féminine en général et par extrapolation, sur les génotypes que vont rencontrer les jeunes filles dès leurs débuts dans la vie sexuelle. Dans le contexte de l'importante population de femmes vivant au Tchad, estimée en 2018 à environ 7.600.000 (INSEED 2014), nos observations épidémiologiques représentent un argument puissant en faveur de l'introduction du vaccin Gardasil-9® dans le programme national de vaccination afin de renforcer la prévention du cancer du col utérin.

Un autre aspect important dans cette analyse de l'épidémiologie moléculaire de l'infection à HR-HPV chez les femmes tchadiennes est l'impact important de l'infection à VIH, qui, bien que n'étant que faiblement présent (3,5%), constituait le principal facteur de risque fortement associé avec le portage génital en HPV chez ces femmes. En effet, les femmes infectées par le VIH étaient 13 fois plus à risque d'avoir une infection génitale à HR-HPV que les femmes séronégatives. Ces résultats sont tout à fait cohérents avec les études antérieures démontrant l'effet aggravant du VIH sur l'épidémiologie infectieuse des HR-HPV et les cancers associés chez les femmes et en particulier dans le contexte africain (Akorolo-Anthony et al., 2013; Ezechi et al., 2014; Adebamowo et al., 2017).

En sommes, nos observations suggèrent, de façon générale, que les femmes vivant au Tchad constituent un groupe vulnérable et négligé, à très haut risque pour l'infection génitale à HR-HPV et par conséquent pour le cancer du col de l'utérus. La très forte prévalence en infection à HR-HPV chez ces femmes démontre clairement que l'infection génitale à HR-HPV au Tchad constitue un problème majeur de santé publique qui reste largement méconnu. Il est donc urgent de mettre en œuvre un programme de prévention du cancer du col utérin au Tchad.

Selon Mortier et al. (Mortier et al., 2016), le dépistage du cancer du col utérin basé sur la cytologie est réalisable chez les femmes au Tchad avec des techniques visuelles peu coûteuses et faciles à interpréter, et pourraient être intégrés aux structures de soins de santé existantes (Mortier et al., 2016). En effet, pour ces femmes porteuses d'une infection à HR-HPV, seule la prévention secondaire avec un dépistage régulier des lésions précancéreuses par cytologie et le suivi de la persistance virale par test moléculaire HPV restent la seule alternative pour prévenir la progression de l'infection vers les stades pathologiques. Cependant, dans le contexte du Tchad, pays à très faible revenu, il existe une grave pénurie de spécialistes en pathologie rendant ainsi la cytologie conventionnelle inadaptée et renforçant par ailleurs la grande nécessité de mettre en œuvre le test moléculaire de détection des HPV (Mboumba Bouassa et al., 2017). En effet, les tests moléculaires permettant de détecter l'ADN des HPV représentent une excellente alternative à la cytologie pour le dépistage du cancer du col utérin, car ils sont plus sensibles, plus reproductibles et plus faciles à interpréter que la cytologie (Mboumba Bouassa et al., 2017). De plus, le temps de traitement rapide des tests ADN HPV pourrait promouvoir l'approche « *screen and treat* » fortement recommandée par l'OMS (Mboumba Bouassa et al., 2017), permettant ainsi de maximiser tout le soutien médical en une seule visite, ce qui permet d'éviter la perte-de-vue de femmes suivies pour une infection par des HR-HPV. En outre, compte tenu du fait qu'une grande partie des femmes tchadiennes adultes vivent dans des zones rurales isolées, loin de toute structure de santé adéquate, l'auto-

prélèvement de sécrétions génitales, effectué à domicile par les femmes elles-mêmes, pourrait, lorsque couplé à des technologies de dépistage moléculaire peu coûteuses, constituer une alternative pertinente permettant d'accroître la couverture du dépistage (Haguenoer et al., 2014a; Mboumba Bouassa et al., 2017). Ce modèle de stratégie de prévention à plusieurs composantes, qui pourrait également intégrer les structures de prise en charge des personnes vivant avec le VIH, est essentiel pour mieux cibler les femmes adultes vivant au Tchad et exposées au risque d'infection génitale à HR-HPV. Cependant, sans assurance médicale, comme c'est le cas pour la plupart des femmes au Tchad, la prévention secondaire basée sur les tests de diagnostic moléculaire ADN HPV reste encore hors de portée de la plupart des femmes tchadiennes. En conséquence, parallèlement à la stratégie de prévention secondaire adaptée aux femmes adultes tchadiennes, environ 600.000 adolescentes âgées de 10 à 14 ans au Tchad (INSEED 2014) pourraient bénéficier d'un programme national de vaccination avec le vaccin prophylactique Gardasil-9® avant leur entrée dans la vie sexuelle (Mboumba Bouassa et al., 2019).

Dans cette analyse de l'épidémiologie infectieuse des HR-HPV chez les femmes africaines, nous avons également pu bénéficier d'une banque de prélèvements biologiques issus de femmes immigrées africaines de première génération (née en Afrique), adultes (âge moyen : 41,7 ans) non-vaccinées et n'ayant jamais subi un dépistage moléculaire et cytologique de l'infection génitale à HR-HPV et des lésions précancéreuses, fortement infectées par le VIH (74%), et vivant en France depuis en moyenne 10 ans. Nous avons constaté une prévalence particulièrement élevée de l'infection génitale à HPV (68%) chez ces femmes, fréquemment associée à des génotypes à haut-risque (56,0%), avec des fréquences assez similaires, tant chez les femmes infectées par le VIH (70,3%), que chez celles séronégatives pour ce facteur de risque (61,5%). Les prévalences élevées d'infection génitale à HR-HPV observées chez ces femmes africaines semblent peu communes au regard de ce qui est couramment décrit dans la population féminine Française, avec des taux d'infection variant entre 8% à 22,8% chez les femmes adultes asymptomatiques et séronégatives pour le VIH (Riethmuller et al., 1999; Clavel et al., 2001; Boulanger et al., 2004; Pannier-Stockman et al., 2008; Bruni et al., 2010; Monsonogo et al., 2011; Vaucel et al., 2011; Heard et al., 2013a; Haguenoer et al., 2014a; Bruni et al., 2018) et 26,4% chez les femmes vivant avec le VIH (Heard et al., 2015). De même, une vaste enquête menée auprès d'un échantillon de la population de femmes européennes séropositives pour le VIH, comprenant six des principales capitales européennes, a révélé des fréquences d'infection génitale à HR-HPV d'environ 35,0% (Heard et al., 2013b), ce qui est bien inférieur aux taux observés dans notre population étudiée. Ces fortes fréquences d'infection à HR-HPV au sein de populations immigrées de

première génération d'origine africaine ne semblent, toutefois, pas constituer un cas isolé. En effet, plusieurs études réalisées au sein de la population de femmes immigrées de première génération, originaires d'Afrique subsaharienne et vivant en Italie, un pays frontalier de la France, ont également dépeint une situation préoccupante vis-à-vis de l'infection génitale à HR-HPV chez ces femmes. Bien que légèrement inférieures aux prévalences décrites dans notre étude, les taux d'infection génitale à HR-HPV variaient de 35,5% (Tornesello et al., 2007), 44% (Tornesello et al., 2007), 45% (Giovannelli et al., 2009), 55,5% (Tornesello et al., 2007) à 65,4% (Paba et al., 2012) chez les femmes séronégatives pour le VIH et 57,1% chez les femmes infectées par le VIH (Tornesello et al., 2007). De plus, au-delà des populations autochtones, les immigrées africaines de première génération présentent des taux d'infection génitale à HPV plus de 8 fois supérieur à ceux observés (7,8%) dans une large population (n=22 814) de femmes immigrées, principalement d'origine européenne et vivant en Italie (Campari et al., 2015). Ces observations soulignent que les femmes immigrées originaires d'Afrique subsaharienne et résidant en France, quel que soit leur statut VIH, sont au moins deux fois plus infectées par des HPV oncogènes que les femmes nées en France métropolitaine et de façon plus générale, que les femmes nées sur le territoire Européen. D'autres part, les fortes prévalences d'infection à HR-HPV observées chez ces femmes immigrées africaines de première génération tendent à refléter l'épidémiologie infectieuse de leur pays d'origine en Afrique subsaharienne. En effet, les prévalences d'infection à HR-HPV en Afrique peuvent atteindre 46,2% chez les femmes adultes séronégatives pour le VIH et 79,1% chez les femmes vivant avec le VIH (Ogembo et al., 2015; Ginindza et al., 2017; Kelly et al., 2017). En conséquence, bien que les femmes immigrées (africaines ou européennes) vivant dans les pays européens soient généralement plus infectées par les HPV que la population féminine autochtone (Campari et al., 2015; García-Villanueva et al., 2019), les immigrantes africaines adultes de première génération vivant en France, en particulier celles infectées par le VIH, constitueraient une population plus à risque pour le cancer du col de l'utérus que les femmes immigrées d'origine Européenne vivant dans les pays d'Europe occidentale, mais surtout que les femmes nées dans les pays d'Europe occidentale, terre d'accueil de ces immigrantes africaines. Des tendances similaires ont été rapportées concernant le risque de survenue de lésions précancéreuses et des cancers qui était plus élevé chez les femmes provenant de pays de forte prévalence en HPV, en particulier les pays d'Afrique subsaharienne, par rapport aux femmes nées en Italie ou dans d'autres pays d'Europe occidentale (Di Felice et al., 2015). Cependant, la durée du séjour des femmes de notre étude (10 ans en moyenne) ne permet pas de discerner clairement si le profil de prévalence de l'infection génitale à HPV observé dans la présente étude est lié à une

exposition aux virus antérieure à la migration, ou si ce profil est associé à des facteurs survenus au cours de la vie sexuelle de ces femmes après leur arrivée en France. Il a été montré que, bien qu'étant sur le territoire Français, les femmes immigrantes d'origine africaine restent confrontées à des situations sociales et sanitaires souvent précaires contribuant à maintenir élevé le risque d'infections sexuellement transmissibles chez ces femmes (Vignier et al., 2018a; Vignier et al., 2018b). En effet, les femmes africaines vivant en France n'ont pas toujours accès aux structures de santé, en particulier celles infectées par le VIH qui se voient refuser des soins par le personnel de santé à cause de leur statut VIH (Grillo et al., 2012; Vignier et al., 2018b). D'autre part, ces femmes, en arrivant en France, font souvent face à des situations sociales précaires et sont souvent contraintes à avoir des rapports sexuels non consentis en contrepartie d'une faveur sociale, les exposant à un risque élevé d'infection sexuellement transmissibles telles que le VIH et l'infection génitale à HPV oncogènes (Pannetier et al., 2018).

Ainsi il ressort de cette étude que les immigrantes africaines vivant en France, en particulier celles de première génération qui sont généralement non-vaccinées et sous-dépistées en ce qui concerne l'infection à HPV et les cancers associés, font face à une épidémiologie infectieuse à HPV oncogènes très préoccupante. Il est dès lors urgent de mettre en œuvre des interventions de dépistage adaptées aux femmes immigrées d'Afrique subsaharienne qui semblent présenter un risque très élevé de cancer du col utérin. L'amélioration de l'accès au dépistage moléculaire régulier de l'infection à HPV et au tri par frottis constitue probablement la condition nécessaire pour prévenir les cancers génitaux chez les femmes africaines immigrées vivant en France. De nouvelles stratégies telles que l'auto-prélèvement des sécrétions cervicovaginales pour le test moléculaire HPV se sont révélées aussi efficaces que le prélèvement vaginal habituel administré par un clinicien (Haguenoer et al., 2014a, Haguenoer et al., 2014b). En outre, l'auto-prélèvement vaginal est une bonne solution pour atteindre les populations isolées et négligées ayant un accès limité aux établissements de santé dans les pays développés (Viviano et al., 2018). Cette nouvelle stratégie efficace pour les femmes africaines pourrait être adaptée aux femmes africaines de première génération vivant en France. En effet, dans un essai randomisé mené en France, la stratégie d'auto-prélèvement s'était avérée plus efficace et plus rentable que la lettre de rappel couramment utilisée en France, augmentant ainsi la participation au dépistage du cancer du col utérin (Haguenoer et al., 2014b, Viviano et al., 2018). Par conséquent, proposer dans les centres de protection sociale, ainsi que dans les foyers de résidence des populations immigrées d'origine africaine, un dispositif d'auto-prélèvement vaginal à réaliser à la maison, pourrait contribuer à accroître la couverture du dépistage du cancer du col de l'utérus dans

cette sous-population négligée de femmes françaises. En outre, une attention particulière devrait être accordée aux femmes immigrées infectées par le VIH, car le VIH constitue un facteur aggravant de la progression des infections à HPV vers le stade cancéreux (Liu et al., 2018). Enfin, des investigations supplémentaires sont nécessaires pour mieux identifier les barrières freinant le dépistage du cancer du col de l'utérus au sein de la population de femmes immigrées d'Afrique subsaharienne vivant en France, en particulier celles de première génération qui ne sont plus éligibles pour la vaccination, selon les recommandations vaccinales françaises (HAS 2019). S'attaquer aux obstacles empêchant l'accès au dépistage pour les femmes africaines vivant en France pourrait être essentiel pour réduire l'incidence et la mortalité associées au cancer du col de l'utérus dans cette population vulnérable et négligée.

A côté des femmes vivant en Afrique subsaharienne et celles nées en Afrique subsaharienne et vivant en France, les hommes ayant des rapports sexuels avec des hommes (HSH) constituent également un groupe très à risque pour l'infection à HPV et pour les cancers associés (Mboumba Bouassa et al., 2018). Dans le cadre de notre analyse, nous avons également évalué la situation épidémiologique de l'infection à HPV au sein d'une population de jeunes HSH (âge moyen : 23,2 ans), fortement infectés par le VIH (69,1%), et vivant à Bangui, la capitale de la République Centrafricaine (Mboumba Bouassa et al., 2018). La prévalence de l'infection anale à HPV était particulièrement élevée ($\cong 70\%$) dans cette population de HSH de la communauté de Bangui. La fréquence des HR-HPV chez ces HSH était très élevée (82,7%) et sensiblement similaire à celles observées dans des groupes de HSH vivant en Afrique du Sud (58%) et au Nigéria (72%) (Müller et al., 2016; Nowak et al., 2016). D'autres études réalisées en Afrique ont rapporté des taux de HR-HPV un peu plus faibles (de 29% à 56%) que ceux observés chez les HSH de Bangui (Donà et al., 2012; Hu et al., 2013; Cranston et al., 2015; Ren et al., 2017). Seules une étude réalisée au sein d'un groupe de HSH aux États-Unis a montré des taux de HR-HPV (87%) plus élevés que ceux observés dans notre étude (Keglovitz et al., 2017). D'autre part, contrairement à ce qui est couramment décrit chez les HSH à travers le monde, chez lesquels les génotypes majoritairement détectés sont les HPV-16 et HPV-18, qui représentent à eux deux, 80% des cas de cancers anaux chez les HSH (De Vuyst et al., 2009), dans notre population d'étude, c'est plutôt le HPV-35 qui était le génotype majoritairement détecté. Les génotypes HPV-16 et HPV-18 n'étaient présent que dans de faibles proportions. Toutefois, plus des deux-tiers des HSH présentant une infection anale à HPV hébergeaient des génotypes couverts par le vaccin Gardasil-9®. Ces résultats suggèrent premièrement que les HSH vivant en République Centrafricaine, en particulier ceux infectés par le VIH, ont un risque très élevé de développer des cancers anaux associés à l'infection à HR-HPV. Ensuite, à l'instar de l'étude de Nowak et

al. (Nowak et al., 2016) menée au Nigéria, dans laquelle le HPV-35 était également le génotype majoritairement détecté au sein d'une population de HSH infectés par le VIH et vivant au Nigéria, notre étude révèle de même que tous les HSH présentant une infection à HPV-35 étaient également coinfectés par le VIH. Ainsi, ces résultats suggèrent que l'épidémiologie de l'infection anale à HPV chez les HSH vivant en Afrique sub-saharienne, en particulier au sein des HSH infectés par le VIH, pourrait être différente de celle observée chez les HSH non-africains. Il faut noter que ces HSH, à cause de l'infection à VIH dont ils sont sujets, sont très à risque de développer un cancer anal associé aux HPV et en tenant compte de leur âge (23,2 ans en moyenne), ils sont donc éligibles et ont nécessairement besoin d'être vaccinés par le vaccin Gardasil-9®. Le génotype HPV-35 n'étant pas couvert par le vaccin Gardasil-9®, cela sous-entendrait que la vaccination au sein de cette population spécifique pourrait ne pas être complètement efficace. Toutefois, plus des 2/3 des infections détectées dans cette population de HSH vivant à Bangui aurait pu être évitées par la vaccination par le Gardasil-9®. Enfin, nos résultats confirment que l'infection anal HPV constitue un problème de santé publique négligé dans la population des HSH vivant en République Centrafricaine dont une grande majorité vivent avec le VIH. A l'instar des femmes africaines vivant en Afrique et celles ayant émigré vers l'Europe, des stratégies de prévention plus adaptées à ces populations spécifiques, vulnérables et très négligées, doivent être mise en place afin de renforcer la lutte contre l'infection à HPV et les cancers associés.

B. Infection génitale à HPV et réponse immunitaire humorale acquise chez des femmes immigrées africaines vivant en France

Parallèlement à l'analyse moléculaire, nous avons également effectué une analyse immunologique de l'épidémiologie de l'infection à HPV au sein de la population de femmes immigrées africaines vivant en France. L'objectif de cette étude était d'évaluer, sur le plan immunologique, la question de la vaccination prophylactique de rattrapage contre l'infection à HPV comme alternative pour la prévention du cancer du col de l'utérus dans une population vulnérable et très à risque, à savoir, les femmes immigrées africaines de première génération vivant en France, originaires de pays de forte endémicité d'infection à HPV, n'étant plus éligibles pour la vaccination selon les recommandations vaccinales Françaises, n'ayant jamais subi de dépistage moléculaire et cytologique de l'infection à HPV et des lésions précancéreuses, et dont une part importante vit avec le VIH. Pour cela, nous avons évalué la

réponse immunitaire humorale à IgG spécifique, dirigée contre 9 des principaux génotypes oncogènes d'intérêt clinique appartenant aux familles $\alpha 7$ (HPV-18, -45 et -68) et $\alpha 9$ (HPV-16, -31, -33, -35, -52 et -58), aussi bien sur le plan systémique que dans les sécrétions génitales des femmes incluses. Nous avons ensuite analysé ces réponses immunitaires en fonction de la présence ou non d'une infection à HPV chez ces femmes. L'épidémiologie moléculaire de l'infection à HPV au sein de ce groupe de femmes a été décrite et discutée dans le paragraphe précédent (*confer* paragraphe « A » de la discussion). En bref, environ les deux-tiers des femmes avaient une infection à HPV et ont été classées dans le groupe I, tandis qu'un cinquième d'entre elles ne présentait pas de preuve moléculaire (ADN HPV) d'infection courante à HPV et était classé dans le groupe II.

Ainsi, plus de 90% des femmes incluses dans cette étude présentaient des IgG circulantes dirigées contre au moins un des génotypes de HPV à haut-risque appartenant aux familles $\alpha 7/\alpha 9$ ($\alpha 7/\alpha 9$ HR-HPV). De plus la séroprévalence d'IgG anti-HPV était significativement plus élevée chez les femmes présentant une infection génitale courante par rapport à celles non infectées, et ce avec une concordance étroite entre les génotypes détectés dans les sécrétions génitales de ces femmes et la spécificité de la réponse IgG anti-HPV sérique. Les taux élevés de séroprévalence d'IgG dirigées contre les $\alpha 7/\alpha 9$ HR-HPV observés sont probablement le témoin d'une forte exposition génitale aux HR-HPV (antérieure et actuelle) chez ces femmes immigrées africaines vivant en France. En effet, les IgG anti-HPV en circulation peuvent persister longtemps après l'élimination de l'infection à HPV par le système immunitaire de l'hôte et peuvent donc constituer un marqueur d'exposition passée au virus (Dillner et al., 1999, Carter et al., 2000, Beachler et al., 2016, Kelly et al., 2018). De plus, la concordance entre la spécificité antigénique de la réponse IgG systémique et le type de HR-HPV détectés au niveau génital indique qu'il y a eu activation de la réponse immunitaire humorale par l'infection génitale courante (Sasagawa et al., 1998, Beachler et al., 2016, Kelly et al., 2018). Ces profils immunologiques avec des séroprévalences très élevées semblent être communs en Afrique subsaharienne. En effet, des profils séro-épidémiologiques similaires ont également été décrits dans des études précédentes menées chez des femmes infectées ou non par le VIH au Kenya (Mbwana et al., 2007), en Ouganda (Namujju et al., 2011) et en Afrique du Sud (Kelly et al., 2018). Ces taux élevés de séropositivité en IgG anti-HPV chez ces femmes peuvent également être, en partie, dus à l'âge relativement avancé de notre population d'étude comme le rapportent des études antérieures montrant une augmentation de la séroprévalence en IgG anti-HPV en fonction de l'âge (Castro et al., 2014, de Araujo-Souza et al., 2014). Ainsi, le taux élevé d'IgG circulants anti- $\alpha 7/\alpha 9$ HR-HPV observé dans la population étudiée pourrait refléter le maintien prolongé de l'exposition aux

HPV ainsi que le renforcement subséquent de la réponse immunitaire dirigée contre le virus. Un tel niveau élevé de réponse IgG anti-HPV indique probablement une incidence cumulative véritablement accrue des infections à HR-HPV chez ces femmes, ainsi que l'absence de décroissance des taux d'anticorps anti-HPV, contrairement à ce qui est généralement observé avec l'augmentation de l'âge et la baisse du risque d'exposition aux HPV qui s'en suit (Wang et al., 2003).

De manière similaire à ce qui a été observé pour les IgG anti-HPV circulantes, des IgG cervicovaginales anti- $\alpha 7/\alpha 9$ HR-HPV ont été détectés chez une large majorité (> 84%) des femmes de cette étude. De même que pour les IgG circulantes, la séroprévalence des IgG cervicovaginales ne dépendait pas de l'infection à VIH, mais elle était beaucoup plus élevée chez les femmes hébergeant une infection génitale courante à HPV. Cependant, le nombre moyen de spécificités antigéniques observées par femme, ainsi que la concordance entre la spécificité antigénique des IgG et les types de HPV détectés dans les sécrétions génitales de ces femmes étaient plus élevés pour les IgG anti-HPV détectés dans le compartiment systémique que pour celles détectées dans les sécrétions cervicovaginales. En outre, moins de la moitié des femmes hébergeant une infection à HPV courante présentait des réponses concordantes, en ce qui concerne la spécificité antigénique, entre les IgG sériques et génitales et le type de $\alpha 7/\alpha 9$ HR-HPV détecté chez ces femmes. Aussi, on peut déduire de ces résultats que les réponses immunitaires humores, tant systémiques que génitales ne peuvent pas être considérées comme équivalentes en ce qui concerne la réponse IgG spécifique des HPV. Il est généralement admis que les IgG monomériques spécifiques des HPV présentes dans les sécrétions cervicovaginales proviennent, en majorité, du compartiment systémique et qu'elles rejoignent le compartiment génital par transsudation à travers les barrières membranaires, tandis qu'une fraction de ces IgG pourraient également être sécrétées localement à la suite d'une activation des inducteurs locaux de la réponse immunitaire, en réponse à l'adsorption des particules de HPV dans la muqueuse génitale et le développement de lésions cervicales associées (Bontkes et al., 1999, Tjiong et al., 2001, Sasagawa et al., 2003, Passmore et al., 2007). Dans notre étude, les IgG génitales anti- $\alpha 7/\alpha 9$ HR-HPV ne se corrélaient que très modérément aux IgG sériques anti- $\alpha 7/\alpha 9$ HR-HPV, ce qui conforte l'hypothèse selon laquelle la muqueuse génitale est un site inducteur médiocre de la réponse immunitaire, n'induisant qu'un faible taux de séroconversion génitale accompagné par une disparition progressive des IgG génitales après l'élimination de l'infection à HPV (Bontkes et al., 1999, Bierl et al., 2005). De plus, d'autres sites anatomiques tout aussi sensibles aux HPV que la muqueuse génitale, auraient également pu provoquer une réponse immunitaire systémique avec une production d'IgG sériques anti-HPV. En effet, la muqueuse rectale étant un puissant inducteur

de la réponse immunitaire humorale systémique à IgG (Mestecky et al., 2005, Stanley, 2009), une infection anale par les génotypes appartenant aux groupes $\alpha 7/\alpha 9$ pourrait ainsi induire, la sécrétion d'IgG sériques anti- $\alpha 7/\alpha 9$ HR-HPV, sans qu'il y ait détection d'ADN d' $\alpha 7/\alpha 9$ HR-HPV dans les sécrétions génitales.

Dans cette étude, la proportion de femmes séropositives pour plusieurs types de HPV était beaucoup plus élevée que la prévalence de détection d'ADN de HPV dans les sécrétions génitales à l'inclusion, ce qui suggère une exposition antérieure à ces génotypes supplémentaires accompagnée d'une activation cumulative de la réponse immunitaire humorale. Les réactivités croisées des IgG anti-HPV, observées dans les compartiments systémique et génital, seraient probablement le marqueur d'antécédents multiples d'exposition plutôt qu'un marqueur d'une infection courante causée par plusieurs génotypes de HPV à la fois. Ainsi, en excluant les IgG sériques spécifiques des types de HPV détectés par PCR dans leurs sécrétions génitales, les femmes du groupe I (présentant une infection à HPV courante) présentaient des profils de réactivité croisée contre une moyenne de 6,7 autres types de $\alpha 7/\alpha 9$ HR-HPV. De plus, les niveaux de réactivité croisée observées étaient plus élevés chez les femmes du groupe I que chez celles du groupe II (ne présentant aucune infection à HPV). Ces résultats indiquent probablement la présence de plusieurs antécédents d'infections génitales par les $\alpha 7/\alpha 9$ HR-HPV, accompagnés par le contrôle et la suppression effective de l'infection, principalement chez les femmes du groupe I. Ce type de profil sérologique serait très probablement lié à une accumulation de partenaires sexuels au cours de la vie (Dillner et al., 1999, Carter et al., 2000, Beachler et al., 2016, Kelly et al., 2018). Considérant le fait que le développement d'une séroconversion induite par une infection à HPV n'est pas systématique (Carter et al., 2000), qu'il faut souvent plusieurs mois avant que la séroconversion puisse être détectée (Carter et al., 2000), et que la plupart des infections à HPV transitoires ne n'induisent pas de réponse anticorps détectable (Carter et al., 2000), Dillner et al. (Dillner et al., 2007) ont suggéré que les anticorps anti-HPV ne joueraient pas de rôle important dans la protection ou la suppression des infections à HPV, mais ces anticorps ne représenteraient uniquement qu'une cicatrice sérologique ou tout au plus un marqueur d'exposition (Dillner et al., 2007). Cependant, bien que cet aspect de la réponse immunitaire humorale anti-HPV reste toujours débattue (Kelly et al., 2018), des études et méta-analyses plus récentes ont mis en évidence le rôle protecteur modeste assuré, par les anticorps anti-HPV acquis après une infection à HPV, contre des infections ultérieures par le même génotype (Malik et al., 2009, Safaeian et al., 2010, Castellsagué et al., 2014, Beachler et al., 2016, Safaeian et al., 2018). Dans notre étude, les taux élevés d'infection multiple ainsi que les taux élevés de réactivité croisée chez des femmes faiblement infectées supposent que les

IgG sériques et cervicovaginaux anti- $\alpha 7/\alpha 9$ HR-HPV n'auraient pas protégé ces femmes contre les infections subséquentes avec des génotypes phylogénétiquement proches, ce qui indique probablement que si protection il y a, elle ne doit être que modeste, comme indiqué précédemment chez des personnes non-vaccinées positives en anticorps anti-HPV (Palmroth et al., 2010, Scherpenisse et al., 2013).

C. Implications pour la révention des cancers HPV-induits : Adaptation des recommandations internationales de prévention contre les infections à HPV et les cancers associés pour les populations à très haut risque

Dans ce paragraphe, il s'agit de la présentation de possibles implications en santé publique découlant de l'analyse des observations faites concernant la situation épidémiologique de l'infection à HPV au sein des différentes populations étudiées. Dans un premier temps nous aborderons la question de la possibilité de la vaccination de rattrapage comme alternative pour la prévention des cancers associés à l'infection à HPV, pour les personnes à très haut risque, n'étant plus éligibles selon la plupart des recommandations vaccinales internationales. Cet aspect sera ensuite complété par l'implication de l'auto-prélèvement des sécrétions génitales à visée diagnostic pour améliorer la couverture du dépistage de l'infection à HPV, élément crucial pour le triage des individus éligibles pour la vaccination de rattrapage.

1. Profils immunovirologiques et éligibilité pour le rattrapage de la vaccination contre l'infection à HPV

La vaccination HPV par les vaccins prophylactiques actuels a pour but d'induire chez l'individu vacciné une réponse immunitaire humorale spécifique aux génotypes de HPV inclus dans le vaccin, caractérisée par de forts titres en anticorps, généralisée à l'ensemble des points d'entrée possible du virus et susceptible d'être efficace durant toute la vie de l'individu vacciné (Dessy et al., 2008, Harper & DeMars, 2017, Pinto et al., 2018). Un des principaux préalables à l'efficacité de la vaccination prophylactique est le fait que l'individu vacciné ne doit pas être déjà infecté par les génotypes inclus dans le vaccin, au moment de la vaccination (Harper & DeMars, 2017, Pinto et al., 2018). C'est sur ces bases que sont fondées les principales recommandations vaccinales dans la plupart des pays à travers le monde, selon lesquelles, la vaccination prophylactique anti-HPV n'est restreinte qu'à une très petite partie de la population féminine : les jeunes filles de 9 à 14 ans, très tôt avant les rapports sexuels, avec un rattrapage possible jusqu'à 19 ans dans la majorité des pays (OMS 2014, OMS 2017,

HAS 2019, Meites et al., 2019). La vaccination sans distinction de genre est actuellement largement débattue (Wolff et al., 2018; Datta et al., 2019), toutefois, la vaccination anti-HPV chez les hommes est, depuis bientôt une décennie, recommandée pour les HSH jusqu'à l'âge de 26 ans dans plusieurs pays (OMS 2014, OMS 2017, Harper & DeMars, 2017, Pinto et al., 2018, HAS 2019, Meites et al., 2019). Plusieurs essais cliniques réalisés sur des femmes de 25 à 45 ans démontrent que la vaccination prophylactique avec les vaccins HPV actuels est efficace à environ 90% contre l'incidence et la persistance des infections à HR-HPV ainsi que la survenue de lésions précancéreuses de haut grade, plus de 7 ans après la vaccination (Wheeler et al., 2009, Einstein et al., 2014, Skinner et al., 2014, Wheeler et al., 2016). Aussi, sans tenir compte des restrictions liées à l'âge, et en ne s'appuyant que sur les critères virologiques et immunologiques, les recommandations actuelles peuvent ainsi faire l'objet de débat. Dès lors, on pourrait considérer comme éligible à la vaccination de rattrapage anti-HPV, du moins théoriquement, tout individu non infecté par les génotypes de HPV couvert par les vaccins anti-HPV actuels.

Au sein des femmes africaines vivant à N'Djamena, au Tchad, 2 groupes de femmes pouvaient ainsi être constitués, en fonction de leur éligibilité pour un éventuel rattrapage de la vaccination avec le vaccin Gardasil-9®. Ainsi, 22,9% des femmes incluses dans le protocole GYNAUTO présentaient une infection génitale à HPV, dont 68,9% d'entre elles étaient causées par des génotypes de $\alpha 7/\alpha 9$ HR-HPV. En considérant le profil virologique de ces femmes, un rattrapage de la vaccination anti-HPV par le Gardasil-9® semble totalement compromis. Pour ces femmes, la prévention secondaire par les techniques moléculaires et cytologiques de dépistage des infections à HPV et des lésions précancéreuses reste leur seule option (Mboumba Bouassa et al., 2017). A côté des femmes infectées par les HPV, plus des trois-quarts (77,9%) des femmes incluses dans le protocole GYNAUTO, était exempt de toute infection à HPV. En considérant le fait que la plupart des génotypes circulant au sein de la population de femmes adultes au Tchad, est majoritairement composée de génotypes de HR-HPV couvert par le vaccin Gardasil-9® (Mboumba Bouassa et al., 2019), et en dépit de l'âge avancé de ces femmes, une vaccination de rattrapage par le Gardasil-9® serait très efficace dans cette population.

Dans la série de femmes immigrées africaines vivant France, en considérant à la fois les paramètres virologiques et immunologiques, on pouvait distinguer 3 catégories de femmes. La première catégorie comprenait la majorité des femmes de l'étude (68,6%) qui présentaient une infection génitale à HPV dont 11,7% étaient causées par des génotypes à bas-risque oncogène (LR-HPV) et 56,8%, une infection génitale causée par des génotypes $\alpha 7/\alpha 9$ HR-HPV. Toutes ces femmes avaient, à la fois, des profils sériques et génitaux présentant des spectres de

réactivité antigénique d'IgG anti-HPV très larges (sérum : 6,1 génotypes inclus dans le Gardasil-9® et génital : 3,7 génotypes inclus dans le Gardasil-9®), indiquant probablement plusieurs antécédents d'infections génitales par des $\alpha 7/\alpha 9$ HR-HPV. Un rattrapage de la vaccination par le vaccin Gardasil-9® semble être largement compromis pour cette première catégorie de femmes.

La deuxième catégorie était composée des femmes (21,6%) ne présentant pas d'infection courante à HPV, mais tout en étant séropositives en IgG anti-HPV contre une moyenne de 1,4 génotypes inclus dans le vaccin Gardasil-9® au niveau sérique et une moyenne de 1 génotype vaccinal pour les IgG génitales. Un rattrapage de la vaccination par le Gardasil-9® pourrait a priori se révéler efficace chez des femmes présentant de tels profils virologiques et immunologiques.

Enfin, la troisième catégorie comprenait environ 10% des femmes de l'étude chez lesquelles, ni ADN de HPV, ni d'IgG anti-HPV n'ont pu être détectés. Bien que moins nombreuses, ces femmes pourraient pleinement être éligibles pour un rattrapage de la vaccination contre les infections à HPV par le vaccin Gardasil-9®.

En sommes, ces résultats suggèrent premièrement que la vaccination de rattrapage pourrait se révéler être une alternative efficace chez une grande majorité de femmes africaines adultes vivant dans des pays de forte endémicité d'infection à HPV et dans lesquels les stratégies actuelles de prévention contre le cancer du col de l'utérus peinent à porter des fruits. Ensuite cette alternative pourrait également renforcer les stratégies de prévention contre le cancer du col de l'utérus déjà en place dans les pays développés, en particulier pour les populations très à risque comme les femmes immigrées de première génération, originaires de pays de forte endémicité d'infection à HPV, arrivant dans leurs pays d'accueil, pour la plupart, déjà sexuellement actives et n'ayant jamais été vaccinées contre l'infection à HPV, ni subi de dépistage cytologique des lésions précancéreuses, n'ayant pas toujours accès aux structures de santé spécialisée dans leur pays d'accueil, et plus particulièrement celles vivant avec le VIH. Ces déductions sont en adéquation avec les conclusions préliminaires rapportées par plusieurs essais cliniques actuellement en cours (Wheeler et al., 2009, Einstein et al., 2014, Skinner et al., 2014, Wheeler et al., 2016), tel que le programme HPV-FASTER, qui suggère que l'extension des stratégies de vaccination aux femmes adultes (≥ 30 ans) associée à un meilleur accès au dépistage primaire de l'infection à HPV (pour le triage), pourrait considérablement accélérer l'impact sur l'incidence du cancer du col de l'utérus dans le monde (Bosch et al., 2016, Bosch et al., 2018, Stern & Roden 2019). Ces études démontrent que la vaccination de rattrapage pourrait être pleinement efficace ($\cong 90\%$) chez des femmes adultes (25 à 45 ans), même chez celles présentant déjà des preuves sérologiques d'une exposition

antérieure aux HPV (Olsson et al., 2009, Castellsagué et al., 2011). Toutes ces données soulèvent ainsi la question sur la nécessité de mettre à jour les recommandations vaccinales, notamment en étendant l'âge de la vaccination bien au-delà des restrictions actuelles. En octobre 2018, la Food and Drug Administration des États-Unis a étendu la tranche d'âge approuvée pour l'utilisation du vaccin Gardasil-9® de 9 à 26 ans, à 9 à 45 ans (FDA 2018). Enfin, en juin 2019, après avoir examiné les données relatives à la vaccination des adultes contre l'infection à HPV, le comité consultatif sur les pratiques d'immunisation (Advisory Committee on Immunization Practices : ACIP) des États-Unis a mis à jour les recommandations relatives à la vaccination de rattrapage et à la vaccination des adultes plus âgés que la limite fixée pour la vaccination de rattrapage, tout en recommandant la mise en œuvre d'un processus décisionnel clinique pour les personnes âgées de 27 à 45 ans qui pourraient être éligibles pour la vaccination de rattrapage, en fonction de leurs paramètres immunologiques et virologiques (Levy & Downs 2019, Meites et al., 2019).

2. Auto-prélèvement des sécrétions génitales pour le diagnostic moléculaire de l'infection à HPV

Tout comme les programmes de vaccination des jeunes filles, les programmes nationaux de dépistage systématique de l'infection à HPV et des lésions précancéreuses chez les femmes adultes (à partir de 25 ans) en Afrique subsaharienne, peinent à être efficacement mis en place (De Vuyst et al., 2013, Mboumba Bouassa et al., 2017). Ainsi, malgré les nombreux efforts entrepris par les différents acteurs de santé publique internationaux et nationaux pour contrôler le fléau que représente le cancer du col de l'utérus en Afrique subsaharienne, les stratégies de prévention contre l'infection à HPV et le cancer du col de l'utérus, qui ont pourtant été très efficaces dans les pays développés (De Vuyst et al., 2013), peinent à porter des fruits considérables dans la majorité des pays d'Afrique subsaharienne (Mboumba Bouassa et al., 2017, Bosch et al., 2016, Bosch et al., 2018, Stern & Roden, 2019). La mise en place de programmes de vaccination de rattrapage systématique pour les femmes adultes pourrait permettre de renforcer les stratégies déjà en place en Afrique (Bosch et al., 2016, Bosch et al., 2018, Stern & Roden, 2019). Cependant, l'un des principaux facteurs réduisant l'efficacité de cette vaccination tardive étant la présence effective d'une infection courante au moment de la vaccination (présence d'ADN de HR-HPV), il est nécessaire qu'un triage moléculaire soit réalisé en amont de la vaccination afin d'optimiser son efficacité dans le contexte particulier de l'Afrique subsaharienne avec ses fortes prévalences en HPV (Bosch et al., 2016, Bosch et al., 2018, Stern & Roden, 2019). Ainsi, en plus de la vaccination des

jeunes filles et la surveillance cytologique des femmes adultes, la vaccination de rattrapage chez les femmes adultes, après triage moléculaire, combinée au renforcement du dépistage précoce des lésions précancéreuses pourraient s'avérer une alternative efficace pour les pays en voie de développement, permettant de fournir de résultats probants en terme de réduction de l'incidence de nouvelles infections et de cancer avérés, à plus court terme que les stratégies actuelles.

En contexte Africain, un des principaux facteurs handicapant considérablement les programmes de prévention contre le cancer du col de l'utérus, demeure la faible couverture assurée par ces programmes (De Vuyst et al., 2013, Mboumba Bouassa et al., 2017). En effet, en Afrique sub-Saharienne, une part importante de la population n'a pas un accès facile aux structures de santé car vivant dans des zones rurales reculées par rapport aux principales agglomérations où se trouvent les principaux centres de santé spécialisée (De Vuyst et al., 2013, Mboumba Bouassa et al., 2017). Similairement à la situation dans leur pays d'origine, les femmes immigrées africaines vivant en France sont également confrontées à des situations sanitaires délétères (Vignier et al., 2018a, Vignier et al., 2018b). En effet, les femmes africaines vivant en France n'ont pas toujours accès aux structures de santé, en particulier celles infectées par le VIH qui se voient refuser des soins par le personnel de santé à cause de leur statut VIH (Grillo et al., 2012, Vignier et al., 2018b). Bien que la vaccination de rattrapage pourrait constituer, pour toutes ces femmes, aussi bien dans leur pays d'origine que dans leur pays d'accueil, une bonne alternative dans la lutte contre le cancer du col de l'utérus, l'accès aux technologies moléculaires de triage, préalable indispensable à la réussite de cette alternative, s'avère un point crucial à solutionner. Le protocole GYNAUTO nous a permis de montrer que l'auto-prélèvement des sécrétions génitales par les femmes elles-mêmes, sans assistance médicale particulière, avec un voile vaginal, était à la fois bien toléré par les patientes, mais ce dispositif permettait également de fournir des résultats particulièrement intéressant sur la situation infectieuse génitale de ces femmes en parfaite corrélation avec les résultats obtenus à partir de prélèvements standards réalisés par un personnel médical. Ces résultats sont en parfaite adéquation avec des précédentes études également réalisées sur des populations africaines (Haguenoer et al., 2014b, Viviano et al., 2018). La distribution d'un tel dispositif auprès des populations rurales éloignées des centres de santé, lors des campagnes nationales de dépistage de l'infection à HPV, permettrait aux femmes de s'auto-prélever, de conserver le prélèvement à température ambiante avant qu'un coursier médical spécialisé le récupère et l'apporte au centre de santé où le prélèvement sera traité et les analyses moléculaires effectuées. Une telle approche pourrait permettre d'étendre la couverture des programmes de prévention contre l'infection à HPV, et faciliterait l'implémentation de la

vaccination de rattrapage au sein de la population de femmes adultes à risque dans les pays d'Afrique subsaharienne. Cette nouvelle stratégie efficace pour les femmes africaines pourrait être adaptée aux femmes immigrées africaines de première génération vivant en France. En effet, dans un essai randomisé mené en France, la stratégie d'auto-prélèvement s'était avérée plus efficace et plus rentable que la lettre de rappel couramment utilisée en France, augmentant ainsi la participation au dépistage du cancer du col utérin (Haguenoer et al., 2014b, Viviano et al., 2018). Par conséquent, proposer dans les centres de protection sociale, ainsi que dans les foyers de résidence des populations immigrées d'origine africaine, un dispositif d'auto-prélèvement vaginal à réaliser à la maison, pourrait contribuer à accroître la couverture du dépistage du cancer du col de l'utérus dans cette sous-population négligée de femmes françaises.

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Annexes

Annexe 1: Table 2. Principal studies reporting humoral immune response to HPV in serum, genital and saliva samples during natural HPV infection.

First author Year Country	Population	HPV DNA (%)	HPV antigens	Sample	Isotype (%)	Remark
Dillner 1989 (Sweden)	Normal cytology (n=24)	NA	Purified BPV virions	Genital	IgA (25%)	<ul style="list-style-type: none"> • IgA significantly higher in women with CIN than in those with normal cytology • IgA to HPV may be a useful marker of CIN
	Cytological abnormalities (n=18)				IgA (72%)	
Dillner 1993 (Sweden)	Normal cytology (n=30)	HPV DNA positive (n=14): 46.6%	HPV-16-E2-peptide HPV-16-E7-peptide HPV-16-L1-peptide HPV-16-L2-peptide	Serum	IgA-E2 (20.0%) IgA-E7 (0%) IgG-E2 (16.6%) IgG-E7 (63.3%)	Genital IgA against E2 and E7 HPV peptides were more frequently detected in women with genital condyloma than in those harboring normal cytology
	Cytological abnormalities (n=30)	HPV DNA positive (n=5): 16.6%		Genital	IgA-E2 (28.6%) IgA-E7 (17.8%)	
				Serum	IgA-E2 (26.6%) IgA-E7 (13.3%) IgG-E2 (13.3%) IgG-E7 (80.0%)	
				Genital	IgA-E2 (62.1%) IgA-E7 (58.6%)	
Dreyfus 1994 (France)	Normal cytology (n=20)	NA	HPV-16-E2-peptide	Serum	IgA (20%) IgG (20%)	<ul style="list-style-type: none"> • IgA to HPV are correlated between genital and systemic compartment • There was no correlation for IgG to HPV between genital and systemic compartment • IgA to HPV increase significantly with the severity of HPV-related lesions
	Cytological abnormalities (n=40)			Genital	IgA (15%) IgG (15%)	
				Serum	IgA (63.4%) IgG (36.6%)	
				Genital	IgA (48.8%) IgG (46.3%)	
Veress 1994 (Hungary)	Normal cytology (whole cohort) (n=163)	HPV DNA positive (21.5%)	HPV-16-E2-peptide HPV-16-E7-peptide HPV-16-L1-peptide HPV-16-L2-peptide HPV-6/11-L2-peptide	Genital	HPV-16 S-IgA-E2 (19.6%) S-IgA-E7 (3.1%) S-IgA-L1 (6.1%) S-IgA-L2 (2.4%) HPV-6/11 S-IgA-L2 (6.1%)	Genital S-IgA to HPV fluctuated with age during latent HPV infection, with a peak between 25-32 years
Wang 1996 (Sweden)	Normal cytology (n=414)	HPV DNA positive (n=123): 29.7%	HPV-16-L1-VLP HPV-18-L1-VLP HPV-33-L1-VLP	Genital	HPV-16 DNA+ (n=92) HPV-16 IgA (13.4%)	HPV type-restricted IgA antibody response against HPV capsids is detectable in cervical mucus and is associated with concomitant cervical HPV infection
	Cytological abnormalities (n=171)	HPV DNA positive (n=144): 84.2%			HPV-18 DNA+ (n=24) HPV-16 IgA (12.2%) HPV-33 DNA+ (n=27) HPV-16 IgA (12.2%)	
Sasagawa 1998 (Japan)	Normal cytology (n=130)	HPV-16-negative (n=130): 100%	HPV-16-L1-VLP	Serum	IgG (11.5%) IgA (8.5%)	Antibody positivity reflects persistent viral infection that may increase the risk for cervical cancer
	Cytological abnormalities (n=130)	HPV-16-positive (46/130): 35.4%		Serum	IgG (54.0%) IgA (43.0%)	
De Gruil 1999 (The Netherlands)	Normal cytology (n=9)	HPV-16-positive (n=9): 100%	HPV-16-L1-VLP	Serum	IgG (55.6%)	<ul style="list-style-type: none"> • IgG responses were associated with HPV-16 persistence

	CIN (n=82)	HPV DNA negative (n=13)			IgG (7.7%)	and high-grade CIN lesions <ul style="list-style-type: none"> Humoral immune response against HPV-16 L1 appears not sufficient for the control of HPV infection and CIN 			
		HPV DNA positive (n=69)			IgG (26.1%)				
Bontkes 1999 (The Netherlands)	Cytological abnormalities (whole cohort)	HPV-16-negative (n=111)	HPV-16-L1/L2-VLP	Serum	IgG (14.6%)	Systemic IgA correlated with HPV clearance and could be a marker of successful cellular response induced at local lymph nodes			
					IgA (16.3%)				
				Genital	IgG (8.1%)				
		IgA (8.1%)							
		Serum		IgG (38.6%)					
				IgA (4.5%)					
Genital	IgG (13.8%)								
	IgA (20%)								
Hagensee 2000 (USA)	Normal cytology (n=177)	HPV-16-positive (n=46): (16%)	HPV-16-L1-VLP	Genital	IgG (12%)	<ul style="list-style-type: none"> IgG detection strongly associated with HPV DNA detection S-IgA detection strongly associated with detection of CIN Cervical HPV infection and further detection of HPV-specific genital antibodies takes several months 			
	Cytological abnormalities (n=114)				IgA (6%)				
					S-IgA (8%)				
Marais 2000 (South Africa)	FSW HIV- (n=55)	HPV-16: 42%	HPV-16-L1-VLP	Serum	IgG (70%)	Serum IgA to HPV-16 correlated with protection against HPV-16 persistence			
					Genital		IgA (40%)		
	FSW HIV+ (n=57)	HPV-16: 85%		Serum			IgG (10%)		
					Genital		IgA (14%)		
							IgG (68%)		
					IgA (15%)				
					IgG (33%)				
					IgA (22%)				
Tjong 2000 (The Netherlands)	Normal cytology (22)	NA	HPV-16-E7-protein	Serum	No antibody detected	HPV-16 E7-specific IgG were locally produced in women with HPV-16 positive CIN			
	CIN (38)				Genital		No antibody detected		
				Cervical cancer (22)			Serum	No antibody detected	
	Genital				IgG-E7 (13.1%)				
									IgG-E7 (36.4%)
Tjong 2001 (The Netherlands)	Normal cytology (n=22)	HPV-DNA negative	HPV-16/18-E6/E7-peptide	Serum	No antibody detected	A part of cervicovaginal HPV-specific IgG may be locally produced			
	CIN (n=38)	HPV-16 DNA (50%)					Serum	No antibody detected	
				Cervical Cancer (n=21)	HPV-16 DNA (71%)				Genital
	Genital	IgG-HPV-16-E7 (24%)							
									IgG-HPV-16-E6 (29%)
					IgG-HPV-16-E7 (48%)				
Marais 2001 (South Africa)	Normal cytology (n=36)	HPV-16: 43%	HPV-16-L1-VLP	Oral	IgG (13%)	<ul style="list-style-type: none"> In case of CIN: Serum IgG > Serum IgA Oral IgA > Oral IgG 10% without serum antibody had nevertheless oral antibodies 			
	CIN or SCC (n=81)				IgA (8%)				
							Genital	IgG (44%)	
Cameron	HIV-infected	NA	HPV-6/11-VLP	Serum	IgG-HPV6/11	<ul style="list-style-type: none"> HPV antibody detection in 			

2003 (USA)	women (n=150)		HPV-16-VLP	Oral	(55%) IgG-HPV16 (37%)	oral mucosal transudate is well correlated with the presence of HPV antibodies in serum • HPV antibody detection in saliva is weakly correlated with the presence of HPV antibodies in serum	
					Saliva		IgG-HPV6/11 (31%) IgG-HPV16 (19%)
					OMT		IgG-HPV6/11 (19%) IgG-HPV16 (17%)
Sasagawa 2003 (Japan)	Normal cytology (n=501)	HPV DNA positive (n=227)	HPV-16-L1-VLP HPV-18-L1-VLP HPV-31-L1-VLP HPV-45-L1-VLP	Genital	IgG (36.0%)	<ul style="list-style-type: none"> IgA response to HPV was elicited earlier than the IgG response, and the IgG response to HPV was barely induced in preclinical HPV infection. However, once the IgG response was induced, it persisted longer after HPV clearance The mucosal IgA response to HPV reflects current HPV infection, whereas the IgG response may be associated with cervical lesions 	
					IgA (46.0%)		
	IgG (44.4%)						
	IgA (60.3%)						
Rocha-Zavaleta, 2003 (Mexico)	Normal cytology (n=277)	HPV-16 (49.8%)	HPV-16-L1-VLP	Genital	IgG (6.5%)	Mucosal S-IgA and IgG are correlated with HPV infection, regardless cervical abnormalities	
	Normal cytology (n=171)	HPV-16-negative (n=171)			S-IgA (13%)		
	Cytological abnormalities (n=349)	HPV-16 (49.4%)			IgG (0.0%) S-IgA (5.2%) IgG (27.0%) S-IgA (48%)		
Bierl 2005 (USA)	Normal cytology (n=252)	19.1% (HPV-16: 3.6%)	HPV-16-L1-VLP	Serum	NA		Cervical antibody response in highly HPV-16 exposed individuals may be protective
	CIN I/II/III (n=291)	72.5% (HPV-16: 35.2%)	HPV-16-L1-VLP	Genital	NA		
Buchinsky 2006 (USA)	Unknown cytology (n=107)	HPV DNA positive (n=15): (14.1%)	HPV-16-VLP	Serum	IgG (5.6%)		
				Oral	IgG (2.8%)		
				Passmore 2007 (South Africa)	Normal cytology (n=27)	Cervical: Total: 78% HPV-16:18% Oral: Total: 18% HPV-16:0%	HPV-16-L1-VLP
Oral	IgG (29.6%) IgA (59.3%)						
Genital	IgG (61.5%) IgA (44.4%)						
Serum	IgG (54.8%) IgA (41.9%)						
CIN I (n=31)	Cervical: Total: 87% HPV-16:19% Oral: Total: 21% HPV-16:3%	HPV-16-L1-VLP	Oral		IgG (35.5%) IgA (58.1%)		
			genital		IgG (28.6%) IgA (22.6%)		
			Serum		IgG (46.7%) IgA (33.3%)		
			Oral		IgG (37.8%) IgA (64.4%)		
CIN II/III (n=45)	Cervical: Total: 98% HPV-16:42% Oral: Total: 30% HPV-16:0%	HPV-16-L1-VLP	Genital		IgG (45.0%) IgA (53.3%)		
			Serum		IgG (46.7%) IgA (33.3%)		
			Oral		IgG (37.8%) IgA (64.4%)		
			Genital		IgG (45.0%) IgA (53.3%)		
Mbulawa 2008 (South Africa)	Normal cytology (n=20)	89.3% (HPV-16: 32%)	HPV-16-L1-VLP	Serum	IgG (50%) IgA (50%)	Serum and cervical HPV-16 specific neutralizing antibodies correlate with HPV-16 infection, but not with cervical disease	
			HPV-16-L1-VLP		NAbs (45%)		
			PsV-HPV-16		IgG (54.7%) IgA (34.4%)		
	CIN I/II/III (n=64)		HPV-16-L1-VLP	Serum	NAbs (37%)		
			PsV-HPV-16		IgG (42%) IgA (17%)		
	Normal cytology (n=12)		HPV-16-L1-VLP	Genital	HPV-16-L1-VLP		
			HPV-16-L1-VLP				

			PsV-HPV-16		NAbs (8%)	
	CIN I/II/III (n=31)		HPV-16-L1-VLP	Genital	IgG (64.5%)	
			HPV-16-L1-VLP		IgA (12.9%)	
			PsV-HPV-16		NAbs (22%)	

Annexe 2: Table 1. Baseline characteristics of the 253 study adult women living in N'Djamena, in Chad.

Characteristics	Study women (N=253) n (%) [95%CI]*
Age (years)	
All age [mean (SD), range, years]	35.0 (9.9) (25-65)
25-29	80 (31.6) [25.9–37.4]
30-39	66 (26.1) [20.7–31.5]
40-49	69 (27.3) [21.7–32.7]
≥ 50	38 (15.1) [10.6–19.4]
HIV infection	9 (3.5) [1.3–5.8]
HBV infection	19 (7.5%) [4.3-10.8]
HCV infection	8 (3.2%) [1.1-5.3]
Past history of STI	10 (3.9) [1.5–6.3]
Marital status	
Single	15 (5.9) [3.1–8.8]
Living in couple	198 (78.3) [73.2–83.3]
Divorced	28 (11.1) [7.2–14.9]
Widow	10 (3.9) [1.5–6.3]
Occupation	
Student	36 (14.2) [9.9–18.5]
Unemployed	137 (54.2) [48.1–60.3]
Employed	78 (30.8) [25.2–36.5]
Education level	
Never schooled	50 (19.7) [14.8–24.6]
Elementary school	45 (17.8) [13.1–22.5]
High school	81 (32.1) [26.3–37.7]
University	77 (30.4) [24.7–36.1]
Sexual behaviors	
Age at sexual onset [years]	
< 16	76 (30.1) [24.4–35.7]
16-20	142 (56.2) [50.1–62.3]
> 20	35 (13.8) [9.6–18.1]
Number of sexual partners in the life	
One regular partner	208 (82.2) [77.5–86.9]
Several partners [1 to 5]	45 (17.8) [13.1–22.5]
Birth control method	
Condoms	7 (2.5) [0.8-4.9]
Hormonal	17 (6.4) [3.4-9.8]
Intrauterine device	10 (3.8) [1.5-5.9]
Other	33 (12.4) [8.3-16.4]
None	198 (74.4) [68.8-79.9]
Feminine hygiene during menstruation	
Sanitary napkins	243 (90.3) [86.3-93.5]
Tampons	35 (13.0) [9.2-17.1]
Genital toilet	

Vulva toilet	264 (91.8) [96.5-99.6]
Vagina toilet	211 (78.4) [73.1-84.0]
Post-coital toilet with finger	243 (90.3) [86.5-93.8]
HPV DNA detection using endocervical swab**	
HPV DNA	58 (22.9) [17.8-28.1]
HR-HPV DNA	40 (15.8) [11.3-20.3]
Multiple types of any HPV among HPV-positive swabs	16 (27.6) [16.1-39.1]
Multiple types of HR-HPV among HR-HPV-positive swabs	10 (25.0) [11.6-38.4]
Any 9-valent vaccine types*** among HPV-positive swabs	29 (50.0) [37.1-62.9]
9-valent vaccine HR-HPV types among HR-HPV-positive swabs	27 (67.5) [52.9-82.1]
HPV DNA detection using self-collected veil****	
HR-HPV DNA	97 (38.3) [32.4-44.3]
HR-HPV DNA	63 (24.9) [19.6-30.2]
Multiple types of any HPV among HPV-positive veils	42 (43.3) [33.4-53.2]
Multiple types of HR-HPV among HR-HPV-positive veils	16 (25.4) [14.6-36.2]
Any 9-valent vaccine types*** among HPV-positive veils	48 (49.5) [39.5-59.4]
9-valent vaccine HR-HPV types among HR-HPV-positive veils	43 (68.3) [56.8-79.7]

* The frequency of each variable is presented with their 95% confidence interval in brackets;

** HPV testing using endocervical secretions obtained by clinician-collected flocked swab;

*** The 9-valent Gardasil-9® vaccine (Merck & Co. Inc.) is effective against HPV genotypes 6, 11, 16, 18, 31, 33, 45, 52 and 58;

**** HPV testing using cervicovaginal fluid collected from self-administered veil (V-Veil-Up Gyn Collection Device, V-Veil-Up Pharma Ltd.) introduced within the vaginal canal during 60 minutes.

95%CI: 95% confidence interval; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; STI: Sexual transmitted infection; HPV: Human papillomavirus; HR-HPV: High-risk human papillomavirus; SD: Standard deviation.

Annexe 3: Table 5. Correspondence between the 7,084 (= 253 x 28) results obtained from the 253 paired swab-collected and veil-collected genital specimens to detect the 28 HPV genotypes included in Anyplex™ II HPV28 kit (Seegene).

Swab	Veil	LR-HPV									HR-HPV									Possibly oncogenic								Total		
		6	11	40	42	43	44	53	54	70	16	18	31	33	35	39	45	51	52	56	58	59	68	26	61	66	69		73	82
Negative	Negative	244	253	248	240	248	244	245	241	242	244	246	244	250	246	248	246	245	244	244	244	249	245	253	249	244	253	248	249	6,891
Positive	Positive with same genotype*	4	0	2	6	0	4	5	4	5	4	4	6	0	6	3	5	4	5	6	7	4	3	0	0	2	0	4	4	97
Positive	Positive with different genotypes **,a	0	0	0	0	1 ^b	2 ^c	0	0	0	0	0	0	1 ^d	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Negative	Positive	5	0	3	7	3	3	3	8	6	4	3	3	2	1	2	2	4	4	3	2	0	4	0	4	7	0	1	0	84
Positive	Negative	0	0	0	0	2	2	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	7

Annexe 4: Table 3. Number of serum or cervicovaginal IgG seropositivities to $\alpha 7/\alpha 9$ HR-HPV types included in the nonavalent Gardasil-9® vaccine among study women with positive HPV DNA detection in their cervicovaginal secretions (group I; N=35), including women with HR-HPV DNA (group Ia; N=29) and women with LR-HPV DNA (group Ib; N=6), and among women with negative HPV DNA detection in their cervicovaginal secretions (group II; N=16).

IgG seropositivities to $\alpha 7/\alpha 9$ HR-HPV types of Gardasil-9® ^μ [n; (%)]																									
Systemic compartment									Genital compartment									Systemic or genital compartment							
		0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
Group I	Ia	0 (0%)	0 (0%)	1 (3.4%)	0 (0%)	4 (13.8%)	1 (3.4%)	0 (0%)	23 (79.3%)	2 (6.8%)	3 (10.3%)	3 (10.3%)	6 (20.7%)	4 (13.8%)	1 (3.4%)	9 (31.1%)	1 (3.4%)	0 (0%)	0 (0%)	0 (0%)	1 (3.4%)	1 (3.4%)	2 (6.8%)	2 (6.8%)	23 (79.3%)
	Ib	0 (0%)	1 (16.7%)	0 (0%)	0 (0%)	0 (0%)	3 (50%)	2 (33.3%)	0 (0%)	0 (0%)	0 (0%)	3 (50%)	1 (16.7%)	0 (0%)	1 (16.7%)	0 (0%)	0 (0%)	1 (16.7%)	0 (0%)	0 (0%)	1 (16.7%)	0 (0%)	0 (0%)	2 (33.3%)	2 (33.3%)
Group II		6 (37.5%)	5 (31.2%)	5 (31.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	6 (37.5%)	8 (50.0%)	2 (12.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (37.5%)	5 (37.5%)	6 (37.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

PV-16, -18, -31, -33, -45, -52 and -58 are the HR-HPV genotypes targeted by the Gardasil-9® vaccine.

