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To cite this version:

Fabien Taieb. Accès à la charge virale pour les patients infectés par le VIH sous traitement antiretroviral, en zone décentralisée des pays à niveau de ressources faible ou modéré. Médecine humaine et pathologie. Université Montpellier, 2018. Français. NNT : 2018MONTT041 . tel-01972063

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THÈSE POUR OBTENIR LE GRADE DE DOCTEUR
DE L'UNIVERSITÉ DE MONTPELLIER

En Biologie Santé

École doctorale Sciences Chimiques et Biologiques pour la Santé (CBS2)

Unité de recherche : TransVIHMI-Recherches translationnelles sur le VIH et les maladies infectieuses

Accès à la charge virale pour les patients infectés par le VIH sous traitement antiretroviral, en zone décentralisée des pays à niveau de ressources faible ou modéré

Présentée par Fabien Taieb
Le 29 Novembre 2018

Sous la direction de Eric DELAPORTE
et Yoann MADEC

Devant le jury composé de

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UNIVERSITÉ DE MONTPELLIER

RESUME :

Sur le plan mondial, le nombre de patients infectés par le VIH (PVVIH) sous traitement antirétroviral (TARV) est en constante augmentation. Les pays aux ressources limitées (PRL) sont les plus touchés par l'épidémie et de fortes inégalités existent ce malgré des objectifs ambitieux fixés par les Nations Unies en 2015 (90-90-90). Le suivi biologique nécessaire des patients sous TARV est connu et les avantages de la mesure de la charge virale (CV) sont bien établis en termes de détection précoce de l'échec thérapeutique, de détection de la non-observance au TARV et de prévention de l'accumulation de résistances. Cependant, dans les PRL et notamment en zone décentralisée, l'accès à la mesure de la CV reste très restreint. Les obstacles sont divers : difficulté d'accès pour les patients à un laboratoire en capacité d'effectuer cette mesure, manque en ressources humaines et matérielles. L'utilisation des Dried Blood Spot (DBS) comme support de prélèvement permet de surmonter ces barrières notamment du fait de sa simplicité, de l'absence de nécessité de chaine de froid et de l'utilisation des machines existantes.

L'objectif de ce travail de thèse est de proposer et d'évaluer une stratégie afin d'améliorer le suivi virologique des PVVIH sous TARV issus de zones décentralisées de PRL en montrant la faisabilité et la validité d'un système de collecte de DBS afin de donner accès à la mesure de la CV aux patients suivis dans des centres non dotés des machines couteuses et de la logistique nécessaire à la réalisation de celle-ci.

Une méta-analyse sur le contrôle virologique en Afrique Sub-saharienne a mis en évidence un taux de succès virologique en « intention de traiter » de 65.4% [61.8_69.1] et de 56.8% [51.3-62.4] à respectivement 12 et 24 mois de TARV ainsi qu'une proportion de succès significativement plus élevée rapportée dans les essais cliniques comparativement à ceux issus des cohortes.

Ensuite, plusieurs projets ont été menés afin d'obtenir des résultats dans des conditions de vie réelle dans deux zones géographiques différentes: le Cameroun et le Vietnam. Au Cameroun, une étude menée dans 12 sites décentralisés a permis d'évaluer 2215 patients dont 937 ont été évalués virologiquement. Cette étude a montré un faible taux de rétention avec 63.3%, 53.5% et 39.9% à respectivement 12, 24 et 36 mois de l'initiation du TARV. Le taux de suppression virologique était très bas avec 66.6%, 62.7% et 59.8% à respectivement 12, 24 et 36 mois de TARV. Le profil des résistances virales analysé chez les patients ayant une CV>5000 cp/mL a montré une accumulation importante des résistances virales, s'aggravant au cours du temps et hypothéquant les options. Au Vietnam, deux études incluant 198 et 203 patients, ont été menées afin d'évaluer les performances de la mesure de la CV sur DBS comparativement au plasma. Trois techniques de mesure de la CV issues de deux industriels ont été évaluées. Une sensibilité de 93.3% [81.7–98.6], 90.1% [80.7-95.9] et 54.9% [40.3–68.9] a été trouvé avec respectivement l'ancien et le nouveau protocole fournis par Abbott et le nouveau protocole fourni par Roche (FVE). La spécificité retrouvé a été respectivement de 94.8% [90.0–97.7], 96.2% [91.4-98.8] et 100% [97.5–100].

Ainsi, à travers ces travaux, nous avons montré que l'utilisation des DBS est faisable, immédiatement disponible et d'un niveau de performance acceptable. Elle permet d'apporter des données précieuses tant au niveau individuel, collectif que programmatique. L'utilisation des DBS devrait être intégrée à la stratégie d'expansion de l'accès à la CV. Le suivi virologique des patients est un enjeu de Santé Publique majeur dans un contexte d'utilisation en 1ère ligne de TARV de molécules à barrière génétique faible à la résistance, d'augmentation des résistances transmises et acquises et d'un taux d'incidence encore élevé de l'infection.

MOTS-CLES : VIH; Charge virale; DBS; Epidémiologie; Pays à ressources limités

ABSTRACT:

Worldwide, the number of HIV-infected patients (PLWHA) on antiretroviral therapy (ART) is growing steadily. Low- and Middle-Incomes Countries (LMICs) are the most affected and strong inequalities still exist despite ambitious goals set by the United Nations in 2015 (90-90-90). The required biological monitoring of patients on ART is well known and the benefits of viral load (VL) measurement are well established, in terms of early detection of treatment failure, detection of non-adherence to ART and prevention of resistance accumulation. However, in LMICs and especially in decentralized areas, access to VL measurement remains scarce or unavailable. Barriers are various: difficulty for patients to reach a laboratory able to perform this measurement, lack of human and material resources. The use of Dried Blood Spot (DBS) as a sampling carrier allows to overcome these barriers, in particular because of its simplicity, the lack of the necessity of a cold chain and the use of existing machines.

This PhD work aims at proposing and improving virological monitoring of PLWHA on ART living in decentralized areas of LMICs by demonstrating the feasibility and validity of a DBS collection system to provide access to VL measurement for patients followed in centres not equipped with costly machines and the logistics for its realization.

A meta-analysis on virological success in Sub-Saharan Africa was conducted. This work showed a virological success rate in "intention to treat" analysis of 65.4%[61.8-69.1] and 56.8%[51.3-62.4] at 12 and 24 months of ART respectively, and a significantly higher proportion of success reported in clinical trials compared to cohorts.

Then, several projects were conducted to obtain results in real life conditions in two different settings: Cameroon and Vietnam. In Cameroon, a study conducted in 12 decentralized sites evaluated 2215 PLWHA on ART, 937 of whom were virologically evaluated. This study showed a low retention rate with 63.3%, 53.5% and 39.9% at 12, 24 and 36 months after ART initiation, respectively. Virological suppression rate was also low with 66.6%, 62.7% and 59.8% at 12, 24 and 36 months of ART, respectively. Profiles of HIV Drug Resistance (HIVDR) in patients with VL>5000 cp/mL showed a significant accumulation of viral resistance, worsening over time and hypothecating therapeutic options. In Vietnam, two studies, involving 198 and 203 patients, were conducted to evaluate performance of VL measurement on DBS compared to plasma. Three VL techniques from two manufacturers were evaluated. Sensitivities of 93.3% [81.7-98.6], 90.1% [80.7-95.9] and 54.9% [40.3-68.9] were found with the old and optimized protocol provided by Abbott company and the new protocol provided by Roche company (FVE), respectively. Specificities were 94.8% [90.0-97.7], 96.2% [91.4-98.8] and 100% [97.5-100], respectively.

Through this work, we showed that the use of DBS is feasible, immediately available and with an acceptable level of performance. It provides valuable data at the individual, collective and programmatic levels. The use of DBS should be integrated into the strategy of expanding access to VL. Virological monitoring of patients is a major public health challenge in a context of use in first line ART of molecules with a low genetic barrier to HIVDR, an increase of transmitted and acquired HIVDR and a persistently high incidence of infection.

KEY WORDS: HIV; Viral load; DBS; Epidemiology; Limited incomes countries

RESUME VULGARISE en français

Afin de maintenir l'efficacité des traitements contre le VIH, la mesure de la charge virale (CV) est indispensable. Alors que celle-ci est réalisée régulièrement dans les pays riches, son accès reste rare dans les pays pauvres où se trouvent deux tiers des malades. Des contraintes techniques et financières expliquent la faible disponibilité de la CV. Sans CV il impossible d'identifier les patients pour lesquels le traitement n'est pas efficace, chez qui il peut y avoir une apparition de virus résistants plus difficile à traiter. Ce travail vise à montrer, dans des conditions de terrain, que l'utilisation des papiers buvard (DBS) permet de donner accès à la CV. Au Cameroun, les résultats ont montré qu'un nombre important de malades avait une maladie non contrôlée. Au Vietnam, nous avons montré que le DBS permet de bien mesurer la CV. Ainsi, l'utilisation des DBS devrait être intégrée dans une stratégie globale à large échelle afin d'améliorer la prise en charge des malades.

RESUME VULGARISE en anglais

In order to maintain the efficacy of HIV treatment, viral load (VL) measurement is crucial. While it is routinely done in rich countries, its access remains scarce in poor countries where two thirds of patients are living. Technical and financial limitations explain this low availability of VL. Without VL, it is not possible to identify patients for whom the treatment is not fully effective, patients in whom resistant viruses may emerge and are more complicated to treat. This work aims at showing, in field conditions, that the use of Dried Blood Spot (DBS) allows access to VL. In Cameroon, the results showed that a substantial number of patients had an uncontrolled disease. In Vietnam, we have demonstrated that DBS allows acceptable measurement of VL. Therefore, the use of DBS should be integrated into a large-scale global strategy.

REMERCIEMENTS

Mes remerciements s'adressent :

- Au Pr Delaporte, mon directeur de Thèse, qui m'a toujours soutenu dans mes démarches même les moins conventionnelles.
- Au Dr Yoann Madec, mon co-directeur de Thèse, sans qui rien n'aurait été possible. Merci pour tout. Merci pour tes qualités professionnelles et humaines. J'espère que l'aventure ne fait que commencer.
- aux membres du jury : le Pr Bouchaud, le Dr Gallien, le Dr Kouanfack et le Dr Tuaillon.
- A mes Maîtres :

- Le Pr Eric Delaporte pour m'avoir permis de collaborer avec lui depuis 2007, pour le travail accompli à ces côtés, son aide, son soutien, la confiance dont il m'a toujours honoré ainsi que pour son approche de la médecine en particulier dans les pays du Sud.
- Le Pr Bouchaud que je n'ai vu que de loin pour le moment mais qui m'a toujours inspiré un profond respect par son ouverture d'esprit, son approche de la médecine et la multidisciplinarité nécessaire à son exercice plein et entier.
- Le Pr Jean-Ralph Zahar pour ses compétences médicales, microbiologiques et humaines. Son amitié m'honore.
- Le Pr Bertrand Godeau pour l'excellence de son enseignement clinique et théorique. Son approche pragmatique de la médecine interne est pour moi un exemple permanent.
- Le Pr Loirat qui m'a initié remarquablement à une réanimation polyvalente tant sur le plan médical et technique que sur le plan humain.
- Le Pr Cabane qui m'a suivi depuis le début de mes études et toujours soutenu amicalement.
- Le Dr Ellen M. Einterz dont la compétence multidisciplinaire et le dévouement pour l'hôpital de district de Kolofata a toujours forcé mon admiration et mon respect.

- A Mme Odile Gelpi pour m'avoir fait confiance et permis d'intégrer l'Institut Pasteur ainsi qu'au Pr Stanislas Pol pour son écoute et ses conseils.
- A l'ensemble des membres du Centre de Recherche Translationnelle de l'Institut Pasteur et particulièrement aux membres de la coordination clinique dont Cécile, Cassandre ou Anaïs.
- Un remerciement particulier à Mr Mohand Aït Ahmed pour ses compétences professionnelles et humaines. C'est un plaisir quotidien de travailler à ses côtés.
- A l'ensemble des membres de l'unité d'Epidémiologie des Maladies Emergentes de l'Institut Pasteur et une pensée particulière pour Mme Catherine Lemauft.
- A l'ensemble de l'unité TransVIHMI de l'IRD de Montpellier et particulièrement à Martine Peeters.
- A l'ensemble du personnel du laboratoire de virologie IMPM/IRD/CREMER de Yaoundé :

- Le Dr Avelin F. Aghokeng pour son aide précieuse, son encadrement et sa technique.
- Le Colonel Eitel Mpoudi Ngole pour son aide, son soutien et sa gentillesse.
- Mme Nadine Lamare et l'ensemble des autres membres du laboratoire dont Clarisse Tchoumi.

- A Mr Alexis Boupda et l'ensemble du personnel de Care and Health Program à Yaoundé
- Au laboratoire de virologie du National Institut of Hygiene and Epidemiology dont le Pr Tuan Anh Nguyen, le Dr Tram Hong Tran, Tram Pham Phuong, Binh Thanh Nguyen, Nhung Le Hong.
- Au Pr Hien Ho Thi de la Hanoi University of Public Health

• A Mesdames Ellen M. Einterz et Myra Bates qui forcent mon admiration et mon respect pour leur engagement total auprès de leurs malades, leur culture et leur joie de vivre dans un contexte parfois difficile ; elles sont pour moi des exemples et je m'honore de leur amitié.
• A l'ensemble du personnel de l'hôpital de district de Kolofata pour qui j'ai une grande admiration.
Personnellement, mes remerciements s'adressent
• A ma famille :
- ma femme, Johanna, sans qui rien ne serait possible, pour qui j'ai un amour sans faille et une admiration sans limite
- mes enfants Sasha, Selma, Solal et Romane
- ma mère
- mon père
- mes sœurs, leurs conjoints et leurs enfants : Anna, Guillaume, Rachelle et Léon ; Sarah, Sandrine, Clément, Esther, Léna et Samuel
• A ma deuxième famille :
- Marcel, Pascale, Alexis, Laetitia et leurs enfants
- A toute la « bulle » antillaise pleine de vie et d'amour
• A mes proches, mes amis et leur famille :
- Emilio, Dora, Amaya, Violetta et Sandro
- Yann, Karima, Maya et Zakari ; Benjamin, Frédérique, Victor et Balthazar ; et toute la bande de Saint Antoine
- Bertrand, Julie, Macha et Suzon

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Abréviations:

CV :	Charge virale VIH
DBS :	Dried Blood Spot
HSH :	Homme ayant des rapports sexuels avec des hommes
INI :	Inhibiteurs de l'Intégrase
INTI :	Inhibiteurs Nucléosidiques/nucléotidiques de la Transcriptase Inverse
INNTI :	Inhibiteurs Non Nucléosidiques de la Transcriptase Inverse
IP :	Inhibiteurs de la Protéase
NIHE :	National Institute of Hygiene and Epidemiology
OMS :	Organisation Mondiale de la Santé
PHR :	Pays à haut niveau de revenu
POC :	Point Of Care
PRL :	Pays au niveau de ressources modéré ou limité
TARV :	Traitement Antirétroviral
UDI :	Utilisateurs de drogues injectables
VIH :	Virus de l'Immunodéfience Humaine

Introduction

Depuis le début de l'épidémie, l'Organisation Mondiale de la Santé (OMS) estime à plus de 70 millions le nombre de personnes infectées et à environ 35 millions le nombre de décès en lien avec la contamination par le VIH, faisant de celle-ci une des grandes pandémies posant des défis majeurs pour la Santé mondiale (1).

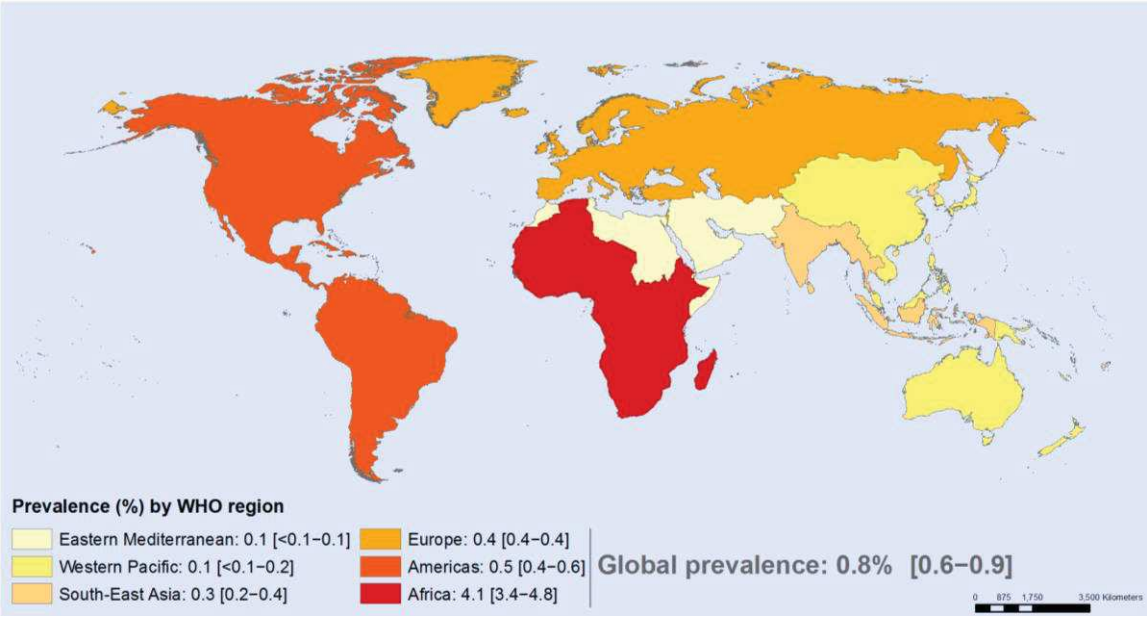

Figure 1 : Prévalence du VIH, selon les régions OMS, chez les adultes âgés de 15 à 49 ans. Source OMS (1)

En 2017, 36,9 millions [31,1-43,9] de personnes dans le monde vivaient avec le VIH dont plus de 25 millions vivent en Afrique subsaharienne. Ce nombre ne cesse de croître du fait d'une baisse de la mortalité grâce à un meilleurs accès aux traitements ainsi qu'à l'efficacité de ces derniers, mais aussi du fait des nouvelles infections dont l'incidence reste trop élevée : 1,8 millions [1,4 million-2,4 millions] de nouvelles infections. Malgré sa diminution, le taux de mortalité reste élevé avec 940 000 [670 000-1,3 million] de décès par an (2). Ainsi, les taux de mortalité et d'incidence persistants à un niveau élevé traduisent un accès encore très imparfait au traitement antirétroviral (TARV).

Dans les pays à haut niveau de revenus (PHR), le traitement antirétroviral (TARV) est fondé sur l'association d'au moins trois molécules hautement actives venant d'au moins deux familles de molécules différentes. Actuellement plus de 20 molécules issues de 6 classes médicamenteuses sont disponibles. Les classes médicamenteuses disponibles sont les inhibiteurs nucléosidiques/nucléotidiques de la transcriptase inverse (INTI), les inhibiteurs non nucléosidiques de la transcriptase inverse (INNTI), les inhibiteurs de la protéase (IP), les inhibiteurs de l'intégrase (INI), les inhibiteurs de la fusion (IF) et les antagonistes du CCR5 (anti-CCR5). En 2018, le TARV de 1ère ligne reste une association de 2 INTI avec un 3ème agent. La mise sous TARV est maintenant recommandée quelque soit le niveau de CD4 (3).

Dans les Pays au niveau de ressources modéré ou limité (PRL) suivant les recommandations de l'OMS , la composition des traitements antirétroviraux (TARV) proposés aux patients est standardisée. En ce qui concerne cet aspect, les recommandations de l'OMS n'ont que peu évolué depuis 2006 et ne sont toujours pas fondées sur le profil de résistance du virus au

niveau individuel. Pour le traitement des adultes, la première ligne de TARV est toujours basée sur la combinaison de 2 INTI et d'un INNTI. Les 2 INNTI recommandés sont toujours l'Efavirenz (EFV) et la Névirapine (NVP). En ce qui concerne les INTI, la Lamivudine (3TC) ou Emtricitabine (FTC) doit être associé au Ténofovir (TDF), à la zidovudine (AZT) (4). La stavudine (d4T) ayant été remplacée par le TDF en 2010 en raison de sa toxicité (5). Un INI, le Dolutégravir (DTG) a été ajouté comme option thérapeutique recommandée en 2016 (4). La seconde ligne standardisée de TARV est toujours composées de 2 INRTs et d'un Inhibiteur de Protéase (IP) boosté par le ritonavir (Lopinavir (LPV/r) ou Atazanavir (ATV/r)). La possibilité d'utiliser le Darunavir boosté (DRV/r) a été introduite en 2016.
Le nombre de patients infectés par le VIH ayant accès à un TARV a très fortement augmenté avec en juin 2017, 20,9 millions [18,4-21,7] de personnes vivant avec le VIH qui accédaient à un TARV, contre 17,1 millions [15,1-17,8] en 2015 et 7,7 millions [6,8-8,0] en 2010. Ceci représentait en 2016, une couverture d'environ 53 % [39 - 65] de toutes les personnes vivant avec le VIH. Cette augmentation a été permise grâce à l'action combinée des plans stratégiques édités par la communauté internationale, de la politique nationale de nombreux pays, des Organisations Non Gouvernementales et de la réduction du prix des TARV.
En effet, à la suite de la conférence internationale de Durban en 2000, et dans le cadre du 6ème Objectif du Millénaire pour le Développement (6, 7), les plans stratégiques et initiatives émanant de la communauté internationale se sont succédés : l'initiative « 3 by 5 » en 2003 : 3 millions de patients sous traitement en 2005 (8) puis « 15 by 15» en 2011 : 15 millions de patients sous traitement en 2015 (9) puis « Ending the AIDS epidemic by 2030 » voté à l'Assemblée Générale des Nations Unies en 2015 (10) et ayant comme première étape le plan « 90-90-90 » : 90% des patients vivant avec le VIH connaissant leur statut sérologique, 90% des patients connaissant leur statut sous TARV, 90% des patients sous TARV en succès virologique, en 2020 (11).

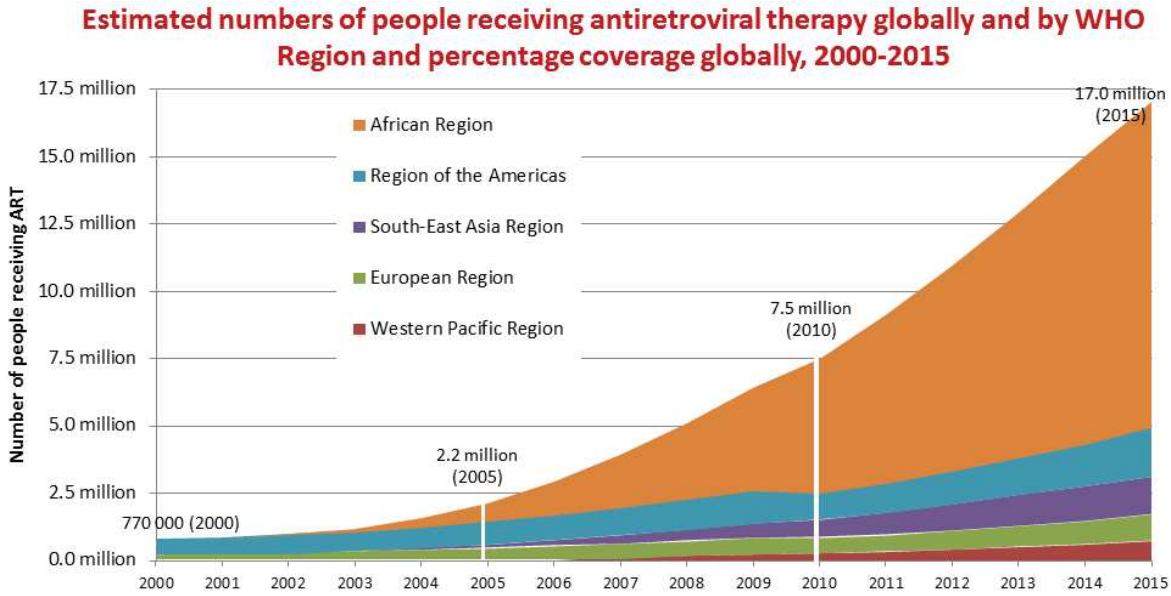

Figure 2 : Estimation du nombre de personne recevant un TARV globalement et selon les régions OMS. Source OMS (12)

L'arrivée des trithérapies antirétrovirales en 1996 a permis au PHR de réduire considérablement le poids de la maladie avec une cassure immédiate de la courbe de

mortalité. Cependant l'accès à ces traitements aux patients issus des PRL n'a pas été immédiat et la cassure de la courbe de mortalité n'a pu être observée qu'à partir de 2005 (2). C'est l'arrivée des médicaments génériques et la décroissance des prix des médicaments à partir de 2000 (passant de plus de 10 000$ à moins de 94$ par personne et par an) (13, 14) qui ont permis d'obtenir, dans de nombreux pays, la gratuité des traitements pour les patients et ce grâce aux efforts conjugués de la communauté internationale et des politiques nationales. De façon concomitante, les formes combinées à dose fixe permettant la prise d'un à deux comprimés par jour ont été mises à disposition des malades.
Enfin, l'évolution des recommandations de mise sous TARV édités par l'OMS, poussée par les stratégies dîtes « Test and Treat » et « Treatment As Prevention » (15-21), ont considérablement élargi le nombre de patients éligibles à la mise sous TARV avec le passage d'un niveau de CD4 indiquant la mise sous traitement de 200/mm^3 en 2006 à 350/mm^3 en 2010 puis la recommandation de mise sous TARV indépendamment du niveau de CD4 en 2015 (5, 22-24).

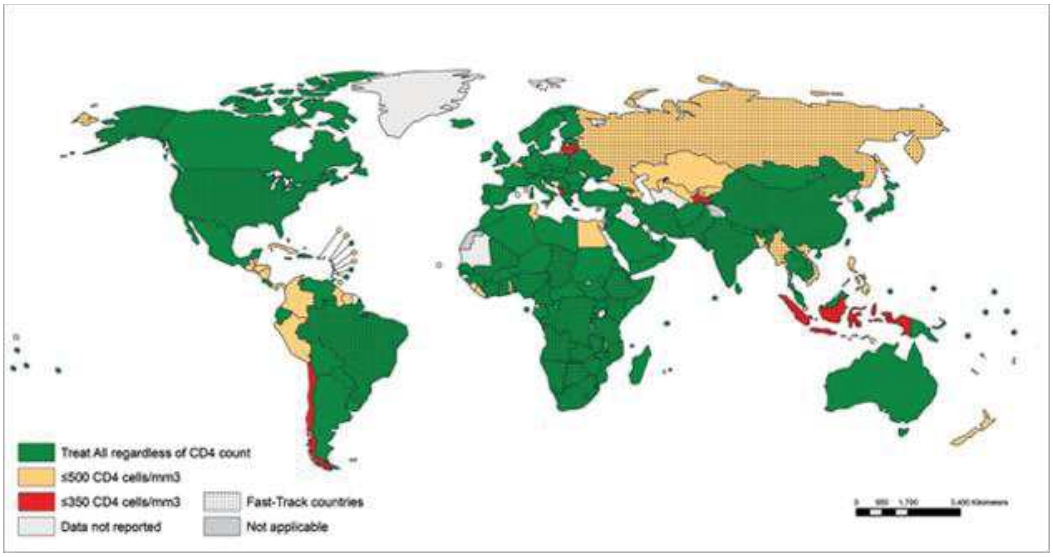

Figure 3 : Mise en œuvre de la politique de l'OMS en matière d'initiation au traitement antirétroviral chez les adultes et les adolescents vivant avec le VIH. (Situation en novembre 2017). Source OMS (25)

Concernant le suivi biologique des patients infectés par le VIH, dans les PHR, le bilan biologique standard est composé à l'initiation du TARV, outre des analyses biologiques standards permettant d'analyser les capacités de tolérance du TARV ainsi que les facteurs de risques cardio-vasculaire et les co-infections potentielles, d'une mesure de la charge virale VIH (CV) et d'un génotypage de résistance à l'initiation du traitement (3). Ces deux dernières mesures permettant une surveillance de sa décroissance dans les premiers mois de mise sous TARV pour la première et une adaptation du TARV aux résistances potentielles du virus pour la dernière. Tout ceci permet de maximiser l'efficacité du TARV donné, sa tolérance et l'observance du patient à celui-ci. Dans le cadre du suivi, une mesure de la CV est recommandée, à l'initiation du TARV, 1 mois, 3 mois puis 6 mois après. L'objectif à 6 mois étant l'obtention de l'indétectabilité de celle-ci (CV<50 cp/mL) puis tous les 3 mois pendant 1 ans puis tous les 6 mois à vie. La mesure de deux CV détectables (CV>200 cp/mL) sur deux prélèvements successifs définit l'échec thérapeutique (échec virologique avéré). En cas d'échec thérapeutique, une mesure des résistances virales est alors recommandée afin de

mettre en place un TARV adapté à ces dernières garantissant ainsi une meilleure efficacité de celui-ci (26).

Pour les PRL, suivant les recommandations de l'OMS, ces dernières ont évolué concernant les modalités de suivi des patients et des définitions de l'échec thérapeutique. Dans le cadre du suivi immunologique (mesure du taux de CD4 sanguin), il était dit « non essentiel » en 2006 puis recommandé tous les 6 mois à partir de 2010. Le suivi virologique (mesure de la charge virale plasmatique) était lui dit « non essentiel » jusqu'en 2010 puis recommandé « si disponible » en 2013 puis recommandé à 6 mois, 12 mois puis tous les ans après la mise sous TARV en 2015.

Les définitions de l'échec thérapeutique ont été tout d'abord fondées sur les critères cliniques (apparition d'un nouveau stade OMS 3 ou 4) puis complétées par des critères immunologiques (CD4< seuil de mise sous TARV ou < 100/mm^3 ou <50% du pic). Quant à la définition virologique de l'échec, le seuil indiquant l'échec est passé d'une CV plasmatique de 10 000 copies/mL en 2006, à 5 000 copies/mL en 2010 puis 1 000 copies/mL en 2013. L'échec thérapeutique étant défini par la mesure de deux charges virales supérieures au seuil recommandé espacées de 3 à 6 mois et après un renforcement de l'observance du patient entre les deux mesures (4, 5, 22-24).

Sur le plan de la mesure des résistances virales, il reste actuellement recommandé un suivi à l'échelle populationnelle tant des résistances acquises que transmises. La mesure à l'échelle individuelle des résistances virales que ce soit en terme surveillance d'acquisition en cours de traitement ou de mesure préalable à l'initiation d'un TARV n'est pas actuellement recommandée (27).

L'ensemble de ces recommandations est principalement fondé sur des réalités économiques et logistiques car les modalités de suivi optimal sont bien connues et correspondent aux recommandations de prise en charge dans les pays à haut niveau de revenu (PHR).

Suite à tous ces efforts, on notait au niveau mondial, une amélioration significative des indicateurs en lien avec les objectifs 90-90-90 fixé en 2015 pour 2020 (28). En effet, en 2016 plus de 2/3 des personnes infectées par le VIH connaissaient leur statut sérologique ; parmi eux 77% [57-89%] avaient accès à un TARV et 82% [60-89%] des patients sous TARV étaient contrôlés sur le plan virologique. Cependant, de nombreuses disparités existent et persistent. L'Afrique de l'Ouest et l'Afrique Centrale notamment sont parmi les régions ayant les moins bons résultats. Fin 2015, les 25 pays d'Afrique de l'Ouest et du Centre comptaient 6,5 millions de personnes vivant avec le VIH, dont 500 000 enfants. La région supportait 18% de la charge de morbidité mondiale du VIH alors qu'elle n'abrite que 6% de la population mondiale. En 2016, 42% [29–54%] des personnes infectées par le VIH connaissaient leur statut sérologique, 35% [24–44%] étaient sous TARV et 25% [18–33%] était virologiquement contrôlés selon les dernières estimations d'UNAIDS (29). Ainsi en 2016, les pays d'Afrique de l'Ouest et Centrale se sont réunis et ont établi un « plan de rattrapage »(30). Ce plan mettait en évidence comme obstacles à l'atteinte des objectifs fixés :

- un engagement politique, une appropriation, une coordination insuffisante ainsi que le faible niveau des financements nationaux en faveur de la riposte au VIH
- des systèmes de santé fragiles, notamment la surmédicalisation des prestations des services liés au VIH
- une décentralisation inadaptée des services de santé, peu ciblée sur la participation et la prestation de services au niveau communautaire tout au long du continuum de soins et de traitements,

- des défaillances des systèmes de gestion des achats et des stocks entraînant de fréquentes ruptures de stock et fragilisant la prestation des services liés au VIH
- une politique largement répandue imposant aux usagers de financer certains frais de santé
- Les niveaux élevés de stigmatisation et de discrimination
- Le nombre réduit de donateurs

Ainsi, l'atteinte des 3 objectifs 90-90-90 relève du surpassement d'obstacles bien différents. Les mesures mises en place tant politiques qu'économiques afin d'atteindre les deux premiers objectifs semblent porter leurs fruits rapidement et ont permis cette augmentation importante du nombre de patients sous TARV. Concernant le troisième objectif, directement impacté par l'amélioration des deux premiers, un obstacle et une condition nécessaire à sa mesure est l'accès au suivi virologique.

Dans ce contexte d'augmentation massive du nombre de patients sous TARV, de l'augmentation de l'espérance de vie donc de la durée de vie sous TARV, l'accès au suivi virologique est devenu primordial et un enjeu de Santé Publique. De plus, il est maintenant bien établi que, dans le cadre de la prise en charge des patients, les critères clinique et immunologique ne permettent pas de détecter l'échec thérapeutique de façon satisfaisante (31, 32). Le suivi virologique doit donc rentrer dans le cadre du suivi standard des soins des patients infectés par le VIH sous traitement dans les pays au niveau de ressources modéré ou limité (PRL), place qu'il occupe depuis des années dans le suivi des patients infectés par le VIH dans les PHR.

Le bénéfice du suivi virologique n'est plus à démontrer tant au niveau individuel que populationnel. Au niveau individuel, la mesure de la charge virale (CV) permet une détection précoce et spécifique de l'échec thérapeutique (33, 34). De plus, cette mesure permet au clinicien de détecter précocement un manque d'observance au TARV de la part du patient et ainsi mettre en place les mesures correctives permettant d'éviter l'échec thérapeutique et la survenue de résistance virale. Il a été démontré que la mesure de la CV et le retour de son résultat au patient permettaient d'améliorer l'adhérence au TARV (35). En effet, grâce à cette mesure objective et compréhensible par le malade et le médecin, des objectifs et une démarche thérapeutique claire et cohérente peuvent être établis. Le suivi virologique permet également une prévention de l'acquisition de résistance virale (36) et une baisse de la morbi-mortalité (37-41).

Au niveau populationnel, la mesure de la CV, en permettant une identification précoce des patients sous TARV ayant une virémie détectable et un renforcement de l'efficacité thérapeutique, participe à la réduction de la transmission du virus via la réduction du niveau de « charge virale communautaire » ainsi qu'à la réduction de la circulation des résistances virales (15, 42, 43). Sur le plan économique, sous certaines conditions de prix et de modalités d'implémentation de la mesure, le caractère cout-efficace a été démontré (37, 38, 44, 45).

Ainsi, la mesure de la CV est un enjeu majeur pour la prise en charge des patients sous TARV dans les PRL, l'optimisation de l'efficacité de ce dernier et la prévention de l'accumulation des résistances virales pouvant compromettre les lignes thérapeutiques futures (46-50).

Le besoin en tests de CV ne fait qu'augmenter et atteindra 30 millions de tests par an d'ici 2020, avec des augmentations de la demande allant jusqu'à 35 % par an. Conscient de cet enjeu, l'ONUSIDA a lancé une initiative mondiale d'accès au diagnostic en 2014 pour renforcer

les partenariats et la coordination des efforts afin d'élargir rapidement l'accès aux outils de diagnostic pour l'intensification du TARV (51).

L'accès la mesure de la CV pour les patients lui n'a que légèrement augmenté entre 2013 et 2015, malgré l'objectif des 90% d'accès d'ici 2020 (52). Ce sont dans les PRL que cet accès reste très limité notamment pour les patients issus des zones décentralisées. Une enquête réalisée par l'OMS dans les PRL a révélé que la couverture globale d'accès à la CV chez les patients sous TARV dans 94 pays était seulement de 23% (53). En Afrique subsaharienne, par exemple, où résident 70 % des patients vivant avec le VIH, plus de 6 millions de personnes sous TARV n'ont pas accès aux tests de CV, et on estime à plus de 10 millions le besoin en CV (52, 54).

Ainsi, un gap important existe entre les besoins en CV et l'accès à cette mesure pour les patients. De façon paradoxale, il est important de noter que l'OMS constatait dans son enquête de 2013 sur des données issues de 39 pays, une importante sous-utilisation des machines existantes avec une médiane de 5 tests virologiques par jour et par machine pourtant capable d'en réaliser plus de 90.

Les obstacles mis en évidence au suivi virologique pouvant expliquer cette sous-réponse aux besoins sont d'ordre i) technique : manque d'infrastructures et d'équipements de laboratoire dans les sites de prises en charge ii) humaine : manque de personnel formé à la technique iii) financier : coût élevé de l'achat et de la maintenance des machines pour les laboratoires et de l'examen pour les patients iv) logistique : difficultés de transport des échantillons nécessitant une chaine de froid et de centrifugation des tubes de sang total afin de réaliser la technique sur plasma, organisation de l'achat et de l'approvisionnement en réactifs source de rupture de stock (55-57). Ces difficultés de transport étant exacerbées pour les zones reculées et notamment pendant les saisons des pluies.

La CV plasmatique est le "gold standard" pour le suivi virologique. Cependant, lorsque le prélèvement sanguin n'est pas réalisé sur le lieu de l'analyse, le transport de ce dernier nécessite une chaine de froid afin d'éviter la destruction de l'ARN viral. De plus, un risque infectieux existe lors du transport de ce type de prélèvement. Dans les PRL, des systèmes de transfert des échantillons plasmatiques ont été mis en place mais ceux-ci sont complexes et onéreux.

Pour faire face à ces difficultés, deux types d'alternatives existent.

La première consiste à l'utilisation des machines dîtes « Point-of Care » (POC). Le test POC n'a pas de définition reconnue universellement. Cependant, les caractéristiques du test semblent reconnues :1) le test doit être réalisé proche du patient à tester 2) le résultat est rendu à la personne testée au cours de la même visite 3) le résultat peut être exploité immédiatement dans le cadre des soins du patient ou pour son éventuel référencement (58). Plusieurs POC fournissant une mesure quantitative de la CV sont actuellement évalués (59-62). Un test POC a été récemment préqualifié par l'OMS (63). Cependant, des obstacles à leur utilisation ont été mis en avant comme le besoin en centrifugeuses électriques pour la séparation du plasma puisque la plupart utilise ce dernier pour obtenir la mesure. Les tests actuels nécessitent toujours, malgré une simplification des procédures par rapport à une mesure conventionnelle de CV par biologie moléculaire, une formation du personnel ainsi que la mise en œuvre de mécanismes de contrôle de la qualité des résultats rendus et de maintenance des machines utilisées (57, 64). De plus, la nécessité d'équiper l'ensemble des sites de prise en charge en vue d'un accès universel à la mesure constitue également un obstacle, ce d'autant que le modèle économique autour de l'utilisation de ces machines n'est pas encore clairement établi avec des coûts non subventionnés des machines et des réactifs peu clairs à l'heure actuelle.

Enfin, cette approche ne permet pas à l'heure actuelle le développement de la surveillance des résistances virales même si des développements sont en cours s'appuyant sur la technique « Sanger » et des détections de mutations ponctuelles (65-67). Cependant, cette approche, si elle aboutit, devra se révéler cout-efficace, d'autant plus qu'elle rajoutera la nécessité de l'achat d'une nouvelle machine au niveau de chaque site de prise en charge.
La seconde alternative consiste en l'utilisation du papier buvard comme outil de transport de prélèvement et notamment de transport de sang total, appelé alors DBS (Dried Blood Spot).

Figure 4: illustration d'un Dried Blood Spot

L'utilisation des DBS, obtenus à partir d'une piqûre au talon ou au doigt permettant de déposer du sang sur un papier filtre, pour la collecte et l'analyse du sang humain remonte au début des années 1960. Le Dr Robert Guthrie a utilisé des échantillons pour mesurer la phénylalanine chez les nouveau-nés afin de détecter la phénylcétonurie (68). Puis son utilisation s'est progressivement étendue à d'autres pathologies et d'autres domaines de recherche étant donné les facilités que cette méthode de prélèvement apporte notamment au niveau de ses modalités de conservation.
Les zones décentralisées des PRL sont caractérisées par leur éloignement géographique par rapport aux laboratoires spécialisés, leur faible voire très faible niveau en ressources médicales et en personnel formé et leur faible développement socio-économique avec un accès à l'eau et l'électricité limité. Certaines sont également caractérisées par leurs conditions climatiques extrêmes avec des températures avoisinant 50°C. Dans ce cadre, la méthode de prélèvement par DBS apparaît être l'outil le plus adapté (69, 70).
Au niveau du site de prise en charge, les DBS sont faciles à confectionner et à conserver car ils ne nécessitent pas de centrifugation du prélèvement, peuvent se conserver à température ambiante et résistent à des conditions de chaleur extrêmes (71, 72). Leur transport vers un site capable de réaliser la mesure de la CV est également aisé car il ne nécessite pas de chaine de froid et peut utiliser le réseau postal existant. De plus, le prélèvement ne représente pas un risque infectieux.
Au niveau du laboratoire capable de réaliser la mesure de la CV, cette dernière sera réalisée en utilisant les machines existantes permettant ainsi d'augmenter et rentabiliser leur utilisation. La réalisation de la mesure de la CV sur DBS ne nécessite pas d'achat de matériel supplémentaire. Enfin, l'augmentation de l'activité des laboratoires permet de renforcer et consolider les capacités du personnel de laboratoire. De plus, le DBS permet de réaliser des mesures de la résistance virale dans le cadre du VIH (73).

Ainsi, dans le cadre de la mesure de la CV, l'utilisation du DBS a été évaluée dans de nombreuses études (74-77). L'OMS a recommandé son utilisation, lorsque la CV plasmatique n'est pas disponible, à partir de 2015 et l'a intégrée dans les dernières recommandations (4,

78). Son utilisation reste pourtant limitée alors que, contrairement à la majorité des POC, la technique est disponible immédiatement.

L'objectif de ce travail de thèse est d'améliorer le suivi virologique des patients infectés par le VIH sous TARV issus de zones décentralisées de PRL en montrant la faisabilité et l'intérêt de la mise en place d'un système de collecte de DBS. Plusieurs projets de recherche opérationnelle ont été menés afin d'obtenir des résultats dans des conditions de vie réelle dans deux zones géographiques différentes ayant des caractéristiques de l'épidémie de VIH différentes : le Cameroun et le Vietnam.

Au Cameroun, l'épidémie de VIH est dite généralisée (79) avec une prévalence estimée en 2016 à 3,8% chez les adultes âgés de 14 à 49 ans (80). Le Cameroun reste l'un des pays de l'Afrique Centrale le plus touché par l'épidémie (29). La distribution géographique de l'épidémie est hétérogène, avec des prévalences élevées dans les régions Centre; Est, Nord-Ouest et Sud du pays (81). Le climat au Cameroun climat est tropical, semi-aride dans le nord et humide et pluvieux dans le reste du pays. La température peut atteindre plus de 45°C au Nord du pays et le taux d'humidité peut être très élevé notamment dans le Sud-Est du pays.

Au Vietnam, l'épidémie de VIH est dite concentrée avec une prévalence du VIH de 0,4% en 2016 (82). L'épidémie affecte principalement les groupes à risque souvent: professionnels du sexe; Homme ayant des rapports sexuels avec des hommes (HSH), utilisateurs de drogues injectables (UDI). Au Vietnam, le climat est subtropical dans le nord et tropical dans le centre-sud, et est influencé par le régime des moussons. Dans le Nord du pays, les températures peuvent monter à plus de 35°C et de fortes précipitations sont constatées de mai à septembre.

Les patients issus des zones décentralisées de ces deux pays n'ont pas actuellement accès à la CV et les obstacles à cet accès sont communs et ceux précédemment évoqués.

Dans un premier temps, j'exposerai un travail de revue systématique de la littérature et de méta-analyse sur le contrôle virologique en Afrique Sub-saharienne permettant de faire un état des lieux de la situation.

Dans un second temps, j'exposerai les résultats obtenus à travers la mise en place des projets.

L'exploitation et le rendu des résultats de la mesure de la CV sur DBS aux patients nécessitant l'assurance de la qualité de ces derniers, une stratégie différente a été adoptée au Cameroun et au Vietnam.

Au Cameroun, le laboratoire en charge de la mesure de la CV (laboratoire IRD/IMPM/CREMER) avait déjà une très large expérience et expertise du DBS (75, 83, 84). Par ailleurs, il est accrédité par l'OMS pour la mesure de la résistance virale HIV sur DBS. Ceci a permis la mise en place d'une évaluation virologique des patients sous TARV en zones décentralisées en utilisant les DBS comme support de prélèvement.

Au Vietnam, le laboratoire en charge de la mesure de la CV (laboratoire de virologie du département VIH/SIDA du National Institute of Hygiene and Epidemiology (NIHE) de Hanoï) n'avait jamais réalisé de mesure de la CV sur DBS. Aussi, une première phase permettant de s'assurer de la qualité des résultats rendus était indispensable. J'exposerai les résultats de deux études comparant les résultats obtenus sur DBS comparativement avec ceux obtenus sur plasma et utilisant 3 techniques de mesure de la CV sur DBS différentes.

J'exposerai ensuite la description des patients issus de la cohorte que nous avons mis en place dans 6 provinces du Nord Vietnam.

Le projet dans son ensemble a été appelé MOVIDA pour Monitoring Of Viral load In Decentralized Area.

Virological success after 12 and 24 months of antiretroviral therapy in sub-Saharan Africa: Comparing results of trials, cohorts and cross-sectional studies using a systematic review and meta-analysis. PLoS One. 2017 Apr 20;12(4):e0174767

PLOS | ONE

RESEARCH ARTICLE

Virological success after 12 and 24 months of antiretroviral therapy in sub-Saharan Africa: Comparing results of trials, cohorts and cross-sectional studies using a systematic review and meta-analysis

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Citation: Taieb F, Madec Y, Cournil A, Delaporte E (2017) Virological success after 12 and 24 months of antiretroviral therapy in sub-Saharan Africa: Comparing results of trials, cohorts and cross-sectional studies using a systematic review and meta-analysis. PLoS ONE 12(4): e0174767. https://doi.org/10.1371/journal.pone.0174767

Editor: Rupert Kaul, University of Toronto, CANADA

Received: September 14, 2016

Accepted: March 15, 2017

Published: April 20, 2017

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Data Availability Statement: Relevant data can be found in Figshare, as follows: https://figshare.com/s/942c5e220b758357b703; DOI: 10.6084/m9.figshare.4811500.

Funding: No funding.

Competing interests: No competing interests.

Abstract

Background

UNAIDS recently defined the 90-90-90 target as a way to end the HIV epidemic. However, the proportion of virological success following antiretroviral therapy (ART) may not be as high as the anticipated 90%, and may in fact be highly heterogeneous. We aimed to describe the proportion of virological success in sub-Saharan Africa and to identify factors associated with the proportion of virological success.

Methods

We performed a systematic review and meta-analysis focusing on the proportion of patients in sub-Saharan Africa who demonstrate virological success at 12 and 24 months since ART initiation, as well as at 6 and 36 months, where possible. Programme factors associated with the proportion of virological success were identified using meta-regression. Analyses were conducted using both on-treatment (OT) and intention-to-treat (ITT) approaches.

Results

Eighty-five articles were included in the meta-analysis, corresponding to 125 independent study populations. Using an on-treatment approach, the proportions (95% confidence interval (CI)) of virological success at 12 (n = 64) and at 24 (n = 32) months since ART initiation were 87.7% (81.3–91.0) and 83.7% (79.8–87.6), respectively. Univariate analysis indicated that the proportion of virological success was not different by study design. Multivariate analysis at 24 months showed that the proportion of virological success was significantly larger in studies conducted in public sector sites than in other sites (p = 0.045). Using an ITT approach, the proportions (95% CI) of virological success at 12 (n = 50) and at 24 (n = 20) months were 65.4% (61.8–69.1) and 56.8% (51.3–62.4), respectively. At 12 months, multivariate analysis

showed that the proportion of success was significantly lower in cohort studies than in trials (63.0% vs. 71.1%; p = 0.017). At 24 months, univariate analysis demonstrated that the proportion of success was also lower in cohorts.

Discussion

Regardless of the time following ART initiation, and of the threshold, proportions of virological success were highly variable. Evidence from this review suggests that the new international target of 90% of patients controlled is not yet being achieved, and that in order to improve the virological outcome, efforts should be made to improve retention in care.

Introduction

At the end of 2015, the World Health Organization (WHO) estimated that about 36.7 million people worldwide were living with HIV, with Sub-Saharan Africa the most affected region in the world with 70% of the HIV burden [1].

The 6th Millennium Development Goal called for an unprecedented mobilization to halt and reverse the AIDS epidemic. UNAIDS also set the 90-90-90 target to help end the HIV epidemics (90% of HIV+ diagnosed, 90% of HIV+ treated, 90% of people on treatment achieving supressed viral load). As a result, by the end of 2015, the number of patients receiving antiretroviral therapy (ART) was estimated at more than 15.8 million, an 85% increase since 2010 [2]. This exceeded the WHO goal to provide HIV treatment to 15 million people by the end of 2015 [3]. However, this increase, as well as the recent WHO "treat-all" recommendation [4, 5], challenge the means to evaluate the effectiveness of ART, especially the long-term effectiveness in resource-limited settings.

To enable rapid deployment of ART, many countries have used the WHO public health approach [5], built on the experience of pilot programmes [6], which takes into account the constraints and weaknesses of health systems in low and middle income countries (LMICs): large numbers of patients, limited availability of drugs, and a lack of biological platforms. This approach is characterized by the standardization of 1st and 2nd line ART, the simplification of decision trees and monitoring, the standardization of biological monitoring and the decentralization of care.

In terms of treatment, the preferred choice for adults is a 1st line ART regimen consisting of a backbone of two nucleoside reverse transcriptase inhibitors (NRTI) (tenofovir (TDF) and lamuvidine (3TC) or emtricitabine (FTC)) and one non-nucleoside reverse transcriptase inhibitors (NNRTI) (efavirenz (EFV)) in one daily fixed dose combination. Zidovudine (AZT) may be an alternative to TDF and nevirapine (NVP) may be an alternative to EFV. Since 2010, stavudine (d4T) was replaced by TDF due to its toxicity [7].

The current WHO guidelines for HIV care [4] recommend viral load monitoring at 6 months since ART initiation, at 12 months and then every 12 months. The switch to 2nd line ART is recommended if the confirmed viral load exceeds the threshold of 1000 copies/mL. Although still not widely available in routine care, viral load monitoring is preferred to CD4 count monitoring for the follow-up of HIV patients.

There are numerous studies presenting virological outcomes in patients on ART in sub-Saharan Africa. However the definition of the outcome varies across studies, making the results of individual studies difficult to understand.

Previous systematic reviews of virological outcomes for patients on ART have focused on sub-Saharan Africa and on levels of acquired resistance to antiretroviral drugs [8–10]. In

LMICs, summary estimates of viral suppression at different thresholds, including an intention to treat analysis are lacking. Although reviews have been published on virological outcomes, to our knowledge, no meta-analysis on recent data is currently available. This is important given the recent increase in the number of patients now receiving ART.

It is important to evaluate individual sites and programs in terms of virological outcomes. However, the pooling of data from several studies in a meta-analysis can be used to inform countries in the development of wider policy and public health actions to address the burden of HIV/AIDS and to enable WHO to generate international recommendations.

Current WHO guidelines recommend cross-sectional studies to monitor virological efficacy and resistance to ART [11], or the use of cohort studies to assess one of eight early warning indicators of HIV drug resistance: viral load suppression 12 months after ART initiation [12]. However, these two approaches may lead to very different outcomes.

This review and meta-analysis aim to provide updated data on proportions of virological success in adults on ART in sub-Saharan Africa. The objectives were to estimate the proportions of virological success at time points following the beginning of ART, to compare proportions of virological success between different study designs (clinical trials, cohort studies and cross-sectional studies) and to identify factors explaining the heterogeneity between the reported proportions of virological success.

Methods

We performed a systematic review and meta-analysis in accordance with the Centre for Reviews and Dissemination guidelines [13] and standards of reporting for systematic reviews (PRISMA) [14] (S1 Checklist).

Search strategy

PubMed, EmBase, Scopus, Web Of Science and Cochrane library were searched for all clinical trials, longitudinal cohort studies and cross-sectional studies on proportions of virological success in adults on ART in sub-Saharan Africa, published in any language between January 1, 2009 and September 30, 2014. We restricted the search to the last 5 years to provide estimates that reflect contemporary care. The search instruction was: (((((HIV OR AIDS[Title/Abstract]))) OR HIV/AIDS[Title/Abstract]) OR ((hiv OR AIDS OR HIV/AIDS[MeSH Terms]))) AND (Africa [Title/Abstract] OR Africa[MeSH Terms]) AND ((((antiretroviral OR ART OR HAART[MeSH Terms]))) OR ((antiretroviral OR ART OR HAART[Title/Abstract]))) AND (((virological OR virologic[MeSH Terms])) OR (virological OR virologic[Title/Abstract]))) AND (("2009/01/01"[PDat]: "2014/05/24"[PDat])). We also manually searched the references of relevant articles to identify studies that might have been missed.

The outcomes of interest were the proportions of patients demonstrating virological success at 6, 12, 24 and 36 months since ART initiation, using on-treatment (OT) and/or intention-to-treat (ITT) analysis.

Study selection

We considered studies conducted in sub-Saharan Africa that reported virological outcomes for at least one of the time points of interest, strictly in HIV-1 infected treatment-naïve adults (with the definition of the start of adulthood varying between studies: from 14 to 20 years) on 1st line ART, corresponding to the WHO recommendations at that time in Africa.

Studies that did not report virological outcomes at a specific time point or that only included HIV-2 infected populations, paediatric populations, patients on 2nd line ART, or in which more than 20% of patients received non-conventional ART regimen, were not included.

Studies reporting virological outcomes on less than 20 patients were also excluded, as such small samples were considered unlikely to provide accurate information and were likely subject to significant biases.

All articles were independently reviewed by two of the authors (FT and YM). The titles and abstracts of all identified articles were reviewed to determine the eligibility of full text papers for inclusion. The remaining full-length articles were retrieved and read independently by the two authors to determine whether to include them in the meta-analysis. Results were compared and discrepancies in opinion between authors as to whether studies should be included were resolved by discussion.

Data extraction

To extract relevant data from the selected articles, a standardized collection form was prepared. Data extraction and validity assessment were carried out independently and in duplicate by two of the authors (FT and YM), and any discrepancies resolved by discussion. The authors contacted the corresponding authors of individual studies to retrieve any missing data. The completed forms and resulting electronic database were checked by a third author (AC) for data accuracy and quality.

For clinical trials, each study arm was considered as an independent study and outcomes were reported independently [15–17]. For longitudinal cohort studies and cross-sectional studies, results which were reported by groups were also regarded as independent studies and outcomes were reported independently.

For all studies, we recorded the threshold at which virological success had been defined, the number of patients evaluated, and the number/proportion of patients demonstrating virological success, enabling OT analysis. For ITT analysis, death, loss to follow-up (LTFU), and the switching to second-line ART prior to the virological evaluation were considered as virological failures, while patients who withdrew from the study or who transferred out of the study prior to the virological evaluation were not considered. The ITT result was not included if the proportion of patients not considered for unknown reasons exceeded 40%.

The quality of the studies included in this meta-analysis was assessed based on the STROBE (Strengthening the reporting of observational studies in epidemiology) checklist [18]. Availability of information regarding the description of the study design, the setting, eligibility criteria, details on virological evaluation, sample size calculation, number of participants at each stage of the study, the reasons for non-participation at each stage of the study, the presentation of baseline characteristics of study participants, reporting of the number of patients in virological success and discussion of the limitations of the study were assessed for all studies eligible for inclusion in the meta-analysis.

Statistical analysis

The overall proportion of patients demonstrating virological success was estimated using a random effect model, following the method of DerSimonian and Laird [19], without weight, and using the *metan* command in Stata (Stata Corp, College Station, Texas, USA). Heterogeneity was indicated if the Cochran test was significant at the level of 0.05 and/or if the I-square statistic exceeded 0.50.

The proportion of patients demonstrating virological success was estimated as the number of patients in virological success divided by the number of patients with a viral load measurement (OT analysis), or as the number of patients in virological success divided by the number of patients who should have reached the evaluation time point minus the number of transfers/withdrawal before the evaluation time point (ITT analysis). Substitution to 2nd line ART before

the evaluation time-point was regarded as virological failure. The standard deviation for all proportions was obtained using a Gaussian approximation.

Due to the variability in reporting of the virological thresholds, virological success at <50 copies/mL included all results at 20, 40 and 50 copies/mL thresholds. In the same way, reporting of results at 100, 150, 200, 400 and 500 copies/mL thresholds were combined and considered as virological success at 400 copies/mL. Virological failure was defined as >1000 copies/mL, in accordance with the latest WHO recommendations [4]. We also assessed the proportion of virological failure at 5000 copies/mL, which corresponds to the 2010 WHO recommendations [7].

The effect of the following factors on the level of virological success was investigated: beginning of enrolment (i.e. ART initiation) (≤2005, ≥2006), end of enrolment (≤2005, 2006–2009, ≥2010), study design, study region [20], type of site (public vs. other), number of sites, decentralized setting (if the authors mentioned "decentralized", "tertiary level", "district level" or "rural" in the method section), general HIV population vs. specific population (i.e. HBV or HCV co-infected patients, TB-infected patients, or Kaposi-diagnosed patients), and tracking of LTFU patients (as reported in the study).

We wanted to evaluate whether previous viral load monitoring affected the level of virological success at the time of evaluation. Hence, we defined a binary indicator of previous routine virological evaluation and we included details of viral load monitoring if performed routinely as part of the study.

The effect of factors describing the population at baseline were also investigated once categorised based on the median across all studies: proportion of women, age, proportion of patients in WHO stage 3 or 4, median CD4 level, median viral load level, first line ART (EFV or NVP-based). The effect of all these factors was investigated using meta-regression and p-values were estimated through a permutation test. To ensure that convergence of the permutation test was achieved, we verified that estimates obtained using 10,000, 30,000 and 50,000 iterations were similar. Factors which presented a p-value <0.20 in univariate analysis were considered in the multivariate model. Using a backward stepwise procedure, we identified factors which remained independently and statistically significantly associated with the outcome. To assess the effect of publication bias, funnel plots representing the proportion of patients in a study in virological success and the sample size of the study were examined [21].

For the ITT analysis, we further distinguished two groups within the general population: studies which enrolled all patients without a minimum follow-up duration and studies which enrolled patients with a minimum follow-up of 6 months. This consideration was important as it has been shown that mortality is particularly high within the first 6 months following ART initiation [22, 23].

Results

Selection of studies

We identified 3613 articles reporting on proportions of virological success following ART initiation. After removing duplicate publications and clearly irrelevant publications, we retrieved 365 articles which were reviewed independently for eligibility. 85 articles identified in the original search contributed to the meta-analysis: 20 articles on clinical trials, 50 articles on cohort studies, 14 articles on cross-sectional studies, and one article reported results from both a cohort and a cross-sectional evaluation [24]. Fig 1 indicates the process of study selection.

To evaluate the quality of the 85 articles considered in this meta-analysis, we selected the items from the STROBE statement that we considered relevant (S1 Table).

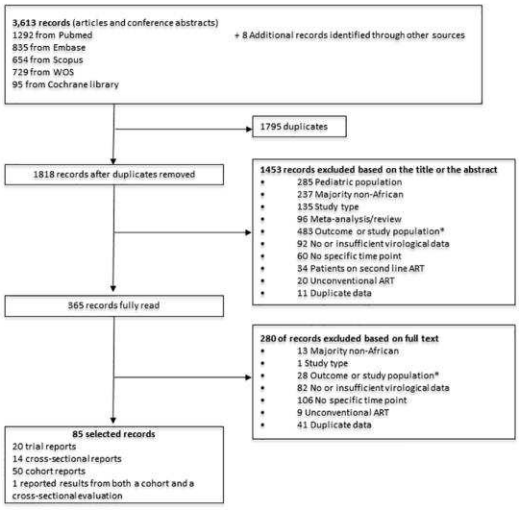

Fig 1. Flow chart of study selection.

https://doi.org/10.1371/journal.pone.0174767.g001

Characteristics of studies

Of the 20 articles on clinical trials included in the review and meta-analysis, two articles reported on the same trial, but reported results at different time points following ART initiation [25, 26]. Among these 19 clinical trials, 8 had a single arm [25, 27–33], 10 had two arms [34–43], and another one had two arms but reported only aggregated results from the two arms [44]. This gave 29 independent study populations. Most trials compared care conditions, and the treatment received in the different study arms were therefore comparable, although some trials considered compared ART regimen.

Of the 15 articles on cross-sectional studies, one was conducted across 5 countries at two different time points in each country [45], three were conducted in one country at two different time points [46–48], two were conducted in one country at one time point but among two different groups [49, 50], two were conducted in one country and at three different time points [51, 52], and the remaining seven were conducted at a single time point [24, 53–58]. This gave 33 distinct study populations.

Of the 51 articles on cohort studies, three articles were based on the same cohort but reported results at different time points [59–61], and two other articles reported on a same cohort [62, 63]. Of the 48 independent cohorts, 31 reported results from the overall cohort [24, 62, 64–92], while 17 reported results from two different groups within the cohort [59, 93–108], although for two of these studies, only one group was considered in our meta-analysis due to sample size restriction [100, 107]. This gave 63 distinct populations.

This meta-analysis is therefore based on 125 distinct populations, which accounted for a total of 156,798 patients, with individual study sizes ranging from 23 to 47,285 patients.

Across the 125 populations, the median proportion of women was 65.7% and individual studies ranged from 0 to 100% proportion of women. The populations were quite homogenous

in terms of age, with the median/mean age in individual studies ranging from 29 to 42 years, and a median age across studies of 36 years. In all but one of the studies, ART initiation followed the WHO guidelines [109]. This study only enrolled patients with CD4 count >350 cells/mm^3 [30]. The median proportion of patients across all studies classified as stage 3–4 according to WHO guidelines was 66.3%. The mean/median CD4 level at ART initiation in individual studies ranged from 33 to 569 cells/mm^3 and the median across all studies was 134 cells/mm^3. Baseline viral load was available for 58 (46.4%) of the study populations. The median viral load level at baseline in individual studies ranged from 4.0 to 6.2 log copies/mL, and across all studies the median was 5.1 log copies/mL. As we only selected studies in which the first line ART fulfilled WHO recommendations, all patients had initiated an ART regimen that contained either 3TC or FTC. For exposure to NNRTI, the median (IQR) proportion of patients across all studies receiving EFV and NVP was 34.5% (1.1–62.5) and 60.0% (31.6–95.0), respectively.

These 125 study populations enabled the evaluation of the proportion of virological success at 6 months (n = 54), 12 months (n = 84), 24 months (n = 41) and 36 months (n = 15) after ART initiation.

Of the 125 study populations, the proportion of virological success was available for using an on-treatment approach in 119 (95.2%). Using an intention-to-treat (ITT) approach, results on the proportion of virological success could be obtained from 76 (60.8%) study populations.

Virological success at 12 months

The results of 84 evaluations of virological success at 12 months after ART initiation were considered: 22 from clinical trials, 47 from cohort studies, and 15 from cross-sectional studies.

Considering OT results only, 64 evaluations of virological success were available at the threshold of 400 copies/mL and the overall proportion (95% confidence interval (CI)) of patients in virological success was 85.3% (83.3–87.3) (Fig 2). The Cochran test ($p<0.001$) and the I-square statistic (96.6%) indicated large between-study heterogeneity.

In univariate analysis, we did not find evidence that the proportions differed by study design (Table 1). The proportions (95% CI) of patients in virological success in clinical trials, cohort studies and cross-sectional studies were 86.1% (82.3–89.8), 85.1% (82.3–87.8) and 85.1% (81.3–88.9), respectively. The proportion of virological success was significantly higher in studies that were set in the public sector, that were in decentralised settings, and that had a higher proportion of patients on EFV-based ART (Table 1). On the other hand, the proportion of virological success was lower in studies in the general population with >6 months of follow-up, compared to studies without this restriction. When combining all factors in a multivariate model, no factor remained independently associated with the proportion of virological failure.

Using an OT approach, 19 evaluations of virological success were available at 40 copies/mL threshold, 22 evaluations of virological success at 1000 copies/mL threshold and 12 evaluations of virological success at 5000 copies/mL threshold. The overall proportions (95% CI) of virological success at these thresholds were 76.3% (71.6–81.0), 87.0% (84.6–89.5) and 90.8% (87.9–93.7), respectively (S1 Fig).

Using an ITT approach, 50 evaluations were considered at the threshold of 400 copies/mL, and the overall proportion of virological success (95% CI) was 65.9% [62.3–69.5] (Fig 3). Again, the Cochran test ($p<0.001$) and the I-square statistic (98.7%) indicated large between-study heterogeneity. In univariate analysis, the proportion of virological success (95% CI) was significantly lower in cohort studies than in clinical trials (63.6% (59.8–67.5) versus 71.1% (64.0–78.2);$p = 0.04$) (Table 2). The proportion of virological success was significantly higher in studies that enrolled patients who had initiated ART more recently (≥2006), and in studies

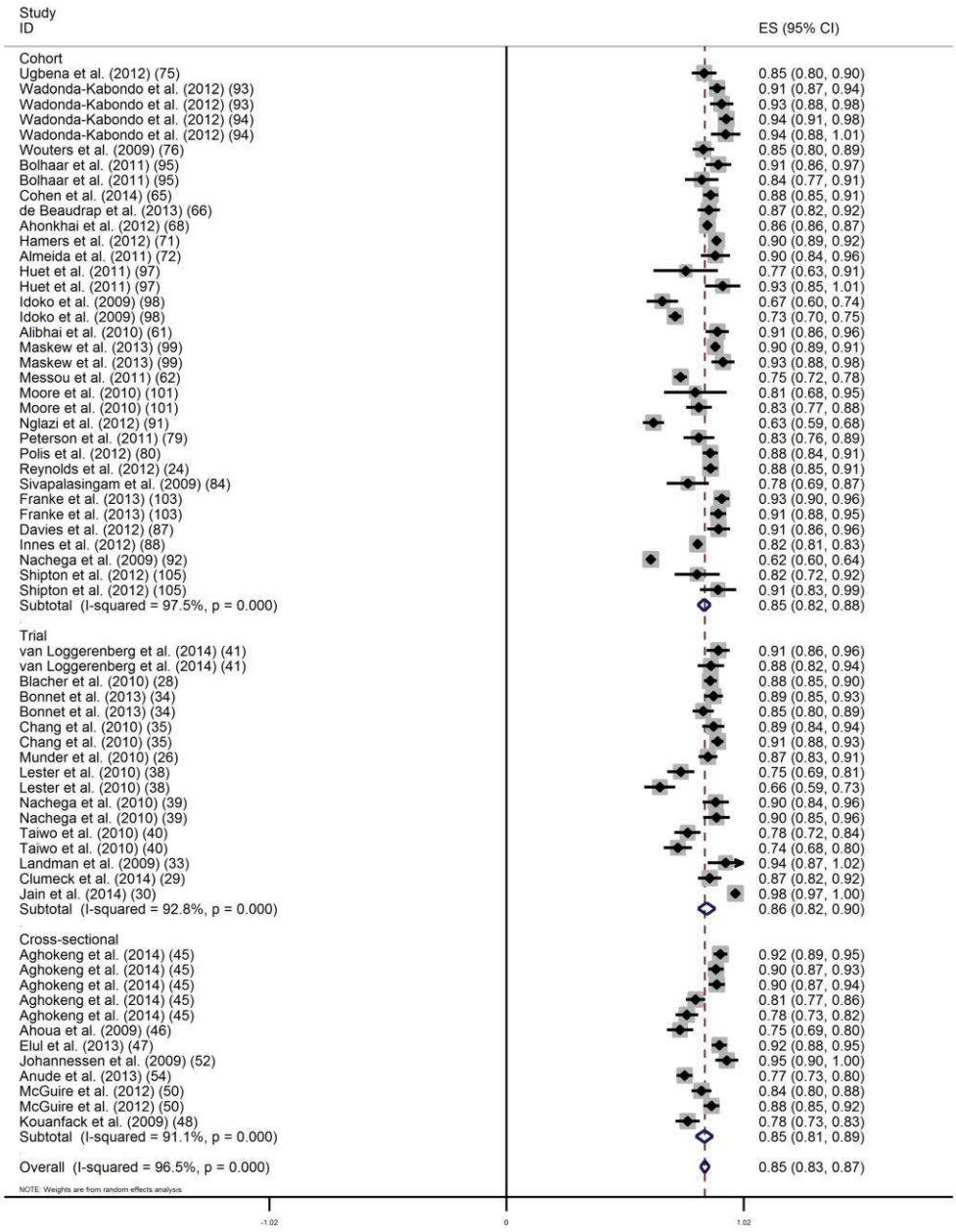
Study
ID
ES (95% CI)
Cohort
Ugbena et al. (2012) (75) 0.85 (0.80, 0.90)
Wadonda-Kabondo et al. (2012) (93) 0.91 (0.87, 0.94)
Wadonda-Kabondo et al. (2012) (93) 0.93 (0.88, 0.98)
Wadonda-Kabondo et al. (2012) (94) 0.94 (0.91, 0.98)
Wadonda-Kabondo et al. (2012) (94) 0.94 (0.88, 1.01)
Wouters et al. (2009) (76) 0.85 (0.80, 0.89)
Bolhaar et al. (2011) (95) 0.91 (0.86, 0.97)
Bolhaar et al. (2011) (95) 0.84 (0.77, 0.91)
Cohen et al. (2014) (65) 0.88 (0.85, 0.91)
de Beaudrap et al. (2013) (66) 0.87 (0.82, 0.92)
Ahonkhai et al. (2012) (68) 0.86 (0.86, 0.87)
Hamers et al. (2012) (71) 0.90 (0.89, 0.92)
Almeida et al. (2011) (72) 0.90 (0.84, 0.96)
Huet et al. (2011) (97) 0.77 (0.63, 0.91)
Huet et al. (2011) (97) 0.93 (0.85, 1.01)
Idoko et al. (2009) (98) 0.67 (0.60, 0.74)
Idoko et al. (2009) (98) 0.73 (0.70, 0.75)
Alibhai et al. (2010) (61) 0.91 (0.86, 0.96)
Maskew et al. (2013) (99) 0.90 (0.89, 0.91)
Maskew et al. (2013) (99) 0.93 (0.88, 0.98)
Messou et al. (2011) (62) 0.75 (0.72, 0.78)
Moore et al. (2010) (101) 0.81 (0.68, 0.95)
Moore et al. (2010) (101) 0.83 (0.77, 0.88)
Nglazi et al. (2012) (91) 0.63 (0.59, 0.68)
Peterson et al. (2011) (79) 0.83 (0.76, 0.89)
Polis et al. (2012) (80) 0.88 (0.84, 0.91)
Reynolds et al. (2012) (24) 0.88 (0.85, 0.91)
Sivapalasingam et al. (2009) (84) 0.78 (0.69, 0.87)
Franke et al. (2013) (103) 0.93 (0.90, 0.96)
Franke et al. (2013) (103) 0.91 (0.88, 0.95)
Davies et al. (2012) (87) 0.91 (0.86, 0.96)
Innes et al. (2012) (88) 0.82 (0.81, 0.83)
Nachega et al. (2009) (92) 0.62 (0.60, 0.64)
Shipton et al. (2012) (105) 0.82 (0.72, 0.92)
Shipton et al. (2012) (105) 0.91 (0.83, 0.99)
Subtotal (I-squared = 97.5%, p = 0.000) 0.85 (0.82, 0.88)
Trial
van Loggerenberg et al. (2014) (41) 0.91 (0.86, 0.96)
van Loggerenberg et al. (2014) (41) 0.88 (0.82, 0.94)
Blacher et al. (2010) (28) 0.88 (0.85, 0.90)
Bonnet et al. (2013) (34) 0.89 (0.85, 0.93)
Bonnet et al. (2013) (34) 0.85 (0.80, 0.89)
Chang et al. (2010) (35) 0.89 (0.84, 0.94)
Chang et al. (2010) (35) 0.91 (0.88, 0.93)
Munder et al. (2010) (26) 0.87 (0.83, 0.91)
Lester et al. (2010) (38) 0.75 (0.69, 0.81)
Lester et al. (2010) (38) 0.66 (0.59, 0.73)
Nachega et al. (2010) (39) 0.90 (0.84, 0.96)
Nachega et al. (2010) (39) 0.90 (0.85, 0.96)
Taiwo et al. (2010) (40) 0.78 (0.72, 0.84)
Taiwo et al. (2010) (40) 0.74 (0.68, 0.80)
Landman et al. (2009) (33) 0.94 (0.87, 1.02)
Clumeck et al. (2014) (29) 0.87 (0.82, 0.92)
Jain et al. (2014) (30) 0.98 (0.97, 1.00)
Subtotal (I-squared = 92.8%, p = 0.000) 0.86 (0.82, 0.90)
Cross-sectional
Aghokeng et al. (2014) (45) 0.92 (0.89, 0.95)
Aghokeng et al. (2014) (45) 0.90 (0.87, 0.93)
Aghokeng et al. (2014) (45) 0.90 (0.87, 0.94)
Aghokeng et al. (2014) (45) 0.81 (0.77, 0.86)
Aghokeng et al. (2014) (45) 0.78 (0.73, 0.82)
Ahoua et al. (2009) (46) 0.75 (0.69, 0.80)
Elul et al. (2013) (47) 0.92 (0.88, 0.95)
Johannessen et al. (2009) (52) 0.95 (0.90, 1.00)
Anude et al. (2013) (54) 0.77 (0.73, 0.80)
McGuire et al. (2012) (50) 0.84 (0.80, 0.88)
McGuire et al. (2012) (50) 0.88 (0.85, 0.92)
Kouanfack et al. (2009) (48) 0.78 (0.73, 0.83)
Subtotal (I-squared = 91.1%, p = 0.000) 0.85 (0.81, 0.89)
Overall (I-squared = 96.5%, p = 0.000) 0.85 (0.83, 0.87)
NOTE: Weights are from random effects analysis
-1.02
0
1.02

Fig 2. On treatment analysis of the proportion of virological success (400 copies/mL) at 12 months of ART by study design.

https://doi.org/10.1371/journal.pone.0174767.g002

with a higher median age at ART initiation. In multivariate analysis, the proportion of virological success remained significantly lower in cohort studies.

Using an ITT approach, 15 evaluations of virological success were available at 40 copies/mL threshold, 12 evaluations of virological success were available at 1000 copies/mL and 3 evaluations of virological success were available at 5000 copies/mL threshold. The overall proportions (95% CI) of virological success at these thresholds were 54.9% (40.9–69.0), 66.5% (60.8–72.2) and 76.9% (71.2–82.6), respectively (S1 Fig).

Virological success at 24 months

Using an OT approach, 32 evaluations of virological success at 24 months after ART initiation at the threshold of 400 copies/mL were available. The overall proportion of virological success (95% CI) was 83.7% (79.8–87.6) (Fig 4). The Cochran test ($p<0.001$) and the I-square statistic (97.8%) indicated large between-study heterogeneity.

In univariate analysis, we did not find evidence that the proportions differed by study design (Table 3). The proportions of virological success (95% CI) in clinical trials, cohort studies and cross-sectional studies were 85.8% (79.1–92.4), 83.9% (78.3–89.5) and 82.1% (76.1–88.1), respectively. The proportion of virological success was found to be significantly higher in public sector sites compared to other sites, in studies with younger populations, and in studies with a higher proportion of women. The proportion of virological success also tended to be higher in studies with lower viral load at ART initiation. When combining all factors in a multivariate model, the only factor that remained significantly associated with a higher proportion of virological success was when the study was set in the public sector.

Using an OT approach, 7 evaluations of virological success were available at 40 copies/mL threshold, 14 evaluations of virological success were available at 1000 copies/mL threshold and 8 evaluations of virological success were available at 5000 copies/mL. The overall proportions (95% CI) of virological success at these thresholds were 83.1% (75.5–90.6), 84.1% (79.2–88.9) and 91.1% (87.3–94.9), respectively (S1 Fig).

Using an ITT approach, 20 evaluations of virological success at 24 months after ART initiation at the threshold of 400 copies/mL were considered. The overall proportion of virological success (95% CI) was 56.8% (51.3–62.4) (Fig 5). Again, the Cochran test ($p<0.001$) and the I-square statistic (98.2%) indicated large between-study heterogeneity. In univariate analysis, the proportion of virological success tended to be higher in clinical trials than in cohort studies (Table 4). The proportion of virological success was significantly higher in studies set in the public sector, and in studies which did not track LTFU patients.

Using an ITT approach, 8 evaluations of virological success at 24 months after ART initiation were available at 40 copies/mL threshold and 6 evaluations of virological success were available at 1000 copies/mL threshold. The overall proportions of virological success (95% CI) were 57.6% (49.6–65.5) and 47.0% (36.1–58.0), respectively. Only one evaluation was performed at the threshold of 5000 copies/mL. This indicated a 67.3% proportion of virological success (95% CI: 63.4–71.2) [24] (S1 Fig).

Virological success at M6

Using an OT approach, 13 evaluations of virological success at 6 months after ART initiation were available at the threshold of 40 copies/mL, 44 evaluations of virological success were available at the threshold of 400 copies/mL, 11 evaluations of virological success were available

Table 1. Virological success (<400 copies/ml) at 12 months–on-treatment analysis.

				Univariate analysis	Multivariate analysis	
Variables	Categories	N	Rate (95% CI)	(p)	Full model (p)	Reduced model (p)
Study design	Cohort	35	85.1 [83.2–87.8]	Ref		
	Trial	17	86.1 [82.3–89.8]	0.90		
	Cross sectional	12	85.1 [81.3–88.9]	0.99		
Beginning of enrolment	≤2005	34	84.4 [81.5–87.4]	Ref		
	≥2006	30	86.3 [83.8–88.8]	0.36		
End of enrolment	≤2005	11	85.6 [82.2–88.9]	Ref		
	2006–2009	45	84.5 [82.2–86.9]	0.92		
	≥2010	7	89.6 [84.9–94.3]	0.46		
Region	Western Africa	16	81.5 [77.4–85.5]	0.11		
	Eastern Africa	25	87.3 [84.8–89.8]	Ref		
	Central Africa	3	85.3 [78.2–92.4]	0.99		
	Southern Africa	16	86.6 [84.1–89.1]	0.99		
	Several region	2	76.2 [0.48–1.00]	0.18		
Type of site	Public sector	51	86.4 [84.5–88.3]	Ref	Ref	Ref
	Other	10	80.2 [73.2–87.3]	0.04	0.76	0.04
Number of sites	1	35	85.1 [81.9–88.3]	Ref		
	≥2	20	85.9 [83.4–88.5]	0.76		
Decentralised setting	No	39	83.9 [80.8–86.9]	Ref	Ref	
	Yes	15	89.6 [85.7–93.5]	0.04	0.35	
	Both	7	82.4 [78.4–86.5]	0.79	0.30	
Population selected	General	44	85.9 [84.3–87.5]	Ref	Ref	
	General, with FU >6 months	7	77.5 [68.9–86.1]	0.01	0.43	
	Specific	7	89.0 [86.5–91.5]	0.44	0.61	
VL monitoring before evaluation	No	31	85.5 [82.1–88.8]	Ref		
	Yes	33	85.2 [82.7–87.6]	0.86		
Tracking of LTFU patients	No	49	85.4 [83.0–87.9]	Ref		
	Yes	15	85.0 [81.1–8.8]	0.85		
Median age at ART initiation (years)	≤36	32	84.7 [82.4–87.0]	Ref		
	>36	26	85.8 [81.4–90.2]	0.60		
Proportion of women	≤65	34	86.8 [83.7–89.9]	Ref		
	>65	30	83.7 [81.3–86.1}	0.14		
Proportion of patients in WHO stage 3–4	≤69	25	84.4 [82.0–86.8]	Ref		
	>69	18	87.9 [85.3–90.6]	0.23		
Median CD4 at ART initiation (cells/mm^3)	≤135	30	85.9 [84.1–87.7]	Ref		
	>135	27	84.4 [79.7–89.1]	0.84		
Median VL at ART initiation (log copies/mL)	≤5.2	14	81.6 [77.3–85.8]	Ref		
	>5.2	22	87.5 [84.8–90.1]	0.09		
Proportion of EFV exposed patients	≤40	22	82.0 [77.2–86.9]	Ref	Ref	
	>40	20	87.4 [84.8–90.0]	0.04	0.99	
Proportion of NVP exposed patients	≤60	23	85.2 [81.4–89.0]	Ref		
	>60	19	83.1 [79.9–86.4]	0.41		

https://doi.org/10.1371/journal.pone.0174767.t001

at the threshold of 1000 copies/mL, and 3 evaluations of virological success were available at the threshold of 5000 copies/mL. The overall proportions (95% CI) of virological success at these thresholds were 71.7% (62.8–80.7), 83.8% (81.0–86.6), 85.5% (81.7–89.3), and 92.7% (87.6–97.8), respectively.

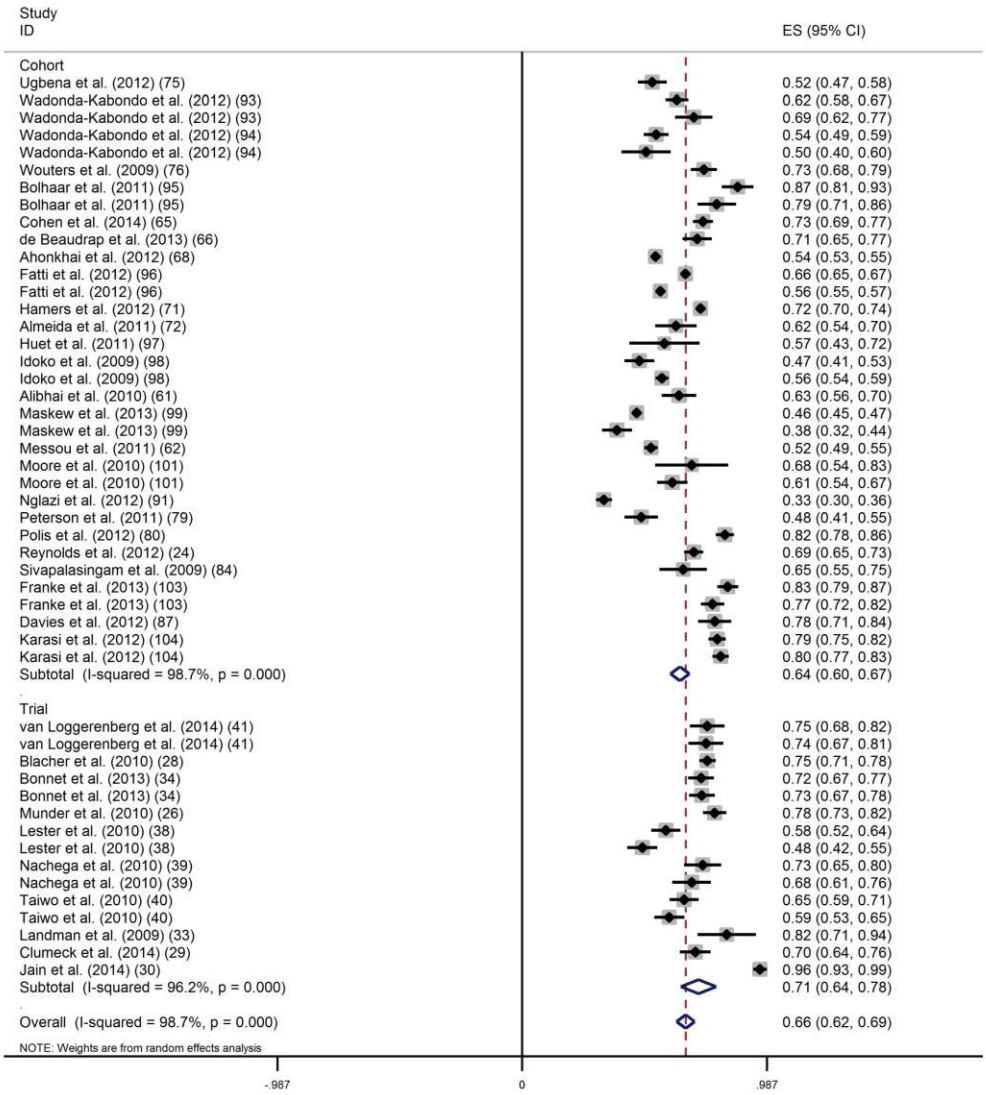

Fig 3. Intention-to-treat analysis of the proportion of virological success (400 copies/mL) at 12 months of ART by study design.

https://doi.org/10.1371/journal.pone.0174767.g003

Using an ITT approach, 7 evaluations of virological success at 6 months after ART initiation were available at the threshold of 40 copies/mL, 31 evaluations of virological success were available at the threshold of 400 copies/mL, 10 evaluations of virological success were available

Table 2. Rate of virological success (<400 copies/ml) at 12 months–intention to treat analysis.

				Univariate analysis	Multivariate analysis	
Variables	**Categories**	**N**	**Rate (95% CI)**	**(p)**	**Full model (p)**	**Reduced model (p)**
Study design	Cohort	35	63.6 [59.8–67.5]	Ref	Ref	Ref
	Trial	15	71.1 [64.0–78.2]	0.037	0.07	0.039
Beginning of enrolment	≤2005	28	62.1 [58.2–66.1]	Ref	Ref	
	≥2006	22	69.4 [63.9–74.9]	0.031	0.82	
End of enrolment	≤2005	7	70.6 [65.5–75.7]	0.22		
	2006–2009	35	62.1 [57.9–66.2]	Ref		
	≥2010	7	74.4 [64.3–84.5]	0.05		
Region	Western Africa	10	58.4 [53.5–63.2]	0.09		
	Eastern Africa	20	68.6 [62.8–74.3]	Ref		
	Central Africa	1	69.9 [63.6–76.1]	1.00		
	Southern Africa	16	62.8 [57.8–67.8]	0.32		
	Several region	1	72.0 [70.3–73.8]	0.99		
Type of site	Public sector	37	65.5 [59.8–71.2]	Ref		
	Other	8	62.4 [57.1–67.7]	0.57		
Number of sites	1	26	67.1 [59.8–74.3]	Ref		
	≥2	16	63.1 [55.6–70.6]	0.36		
Decentralised setting	No	27	66.4 [60.3–70.4]	Ref		
	Yes	11	68.8 [54.5–83.1]	0.80		
	Both	7	58.4 [52.9–63.9]	0.25		
Population selected	General	33	65.1 [60.7–69.4]	Ref		
	General, with FU >6 months	5	59.8 [51.8–67.8]	0.64		
	Specific	6	66.6 [54.3–78.9]	0.96		
VL monitoring before evaluation	No	18	64.4 [60.4–68.5]	Ref		
	Yes	32	66.0 [60.0–72.1]	0.69		
Tracking of LTFU patients	No	36	66.5 [61.2–71.8]	Ref		
	Yes	14	62.7 [57.1–68.4]	0.40		
Median age at ART initiation (years)	≤36	25	59.9 [55.3–64.4]	Ref	Ref	Ref
	>36	18	68.4 [64.2–72.5]	0.029	0.13	0.10
% women	≤65	21	64.7 [58.3–71.1]	Ref		
	>65	27	64.9 [60.7–59.2]	0.94		
% WHO stage 3–4	≤65	15	62.4 [53.5–71.2]	Ref		
	>65	16	68.8 [63.3–74.3]	0.82		
Median CD4 level	≤130	26	63.0 [59.1–66.9]	Ref		
	>130	19	67.5 [61.3–73.6]	0.21		
Median VL level	≤5.2	12	61.3 [53.9–68.8]	Ref		
	>5.2	18	67.2 [61.5–72.9]	0.22		
% with EFV	≤40	15	64.6 [58.6–70.6]	Ref		
	>40	15	65.1 [58.9–71.4]	0.92		
% with NVP	≤60	16	64.3 [58.3–70.2]	Ref		
	>60	14	65.6 [59.7–71.5]	0.80		

https://doi.org/10.1371/journal.pone.0174767.t002

at the threshold of 1000 copies/mL and 2 evaluations of virological success were available at the threshold of 5000 copies/mL. The overall proportions (95% CI) of virological success at these thresholds were 65.0% (48.4–81.6), 68.5% (62.8–74.2), 70.9% (62.7–79.2) and 87.1% (83.6–90.5), respectively (S1 Fig).

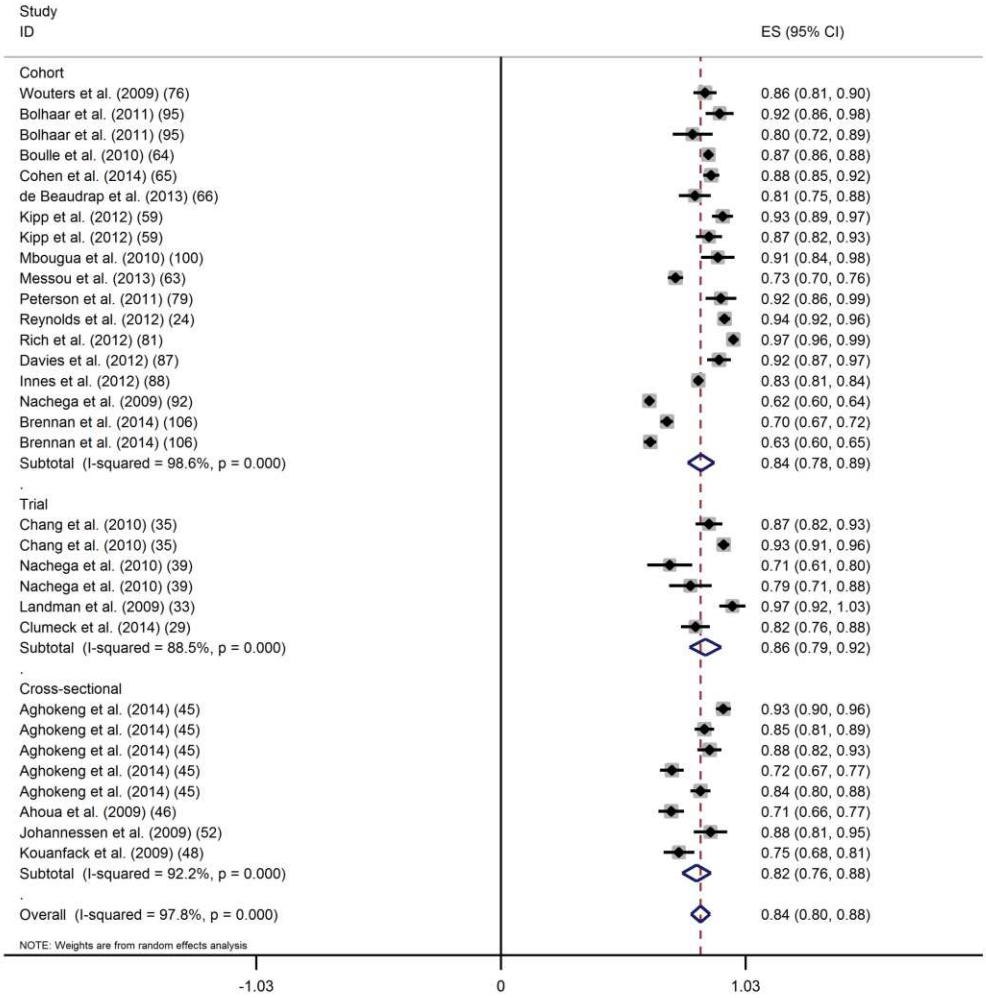

Fig 4. On treatment analysis of the proportion of virological success (400 copies/mL) at 24 months of ART by study design.

https://doi.org/10.1371/journal.pone.0174767.g004

Virological success at M36

Using an OT approach, 12 evaluations of virological success at 36 months after ART initiation were available at the threshold of 400 copies/mL, 4 evaluations of virological success were available at the threshold of 1000 copies/mL, and 3 evaluations of virological success were available at the threshold of 5000 copies/mL. The overall proportions (95% CI) of virological success at these thresholds were 85.8% (82.2–89.3), 81.7% (77.9–85.5) and 92.8% (89.4–96.2), respectively.

Table 3. Rate of virological success (<400 copies/ml) at 24 months–on-treatment analysis.

				Univariate analysis	Multivariate analysis	
Variables	**Categories**	**N**	**Rate (95% CI)**	**(p)**	**Full model (p)**	**Reduced model (p)**
Study design	Cohort	18	83.9 [78.3–89.5]	Ref		
	Trial	6	85.8 [79.1–92.4]	0.88		
	Cross-sectional	8	82.1 [76.1–88.1]	0.94		
Beginning of enrolment initiation	≤2005	21	85.3 [80.6–89.9]	Ref		
	≥2006	11	80.8 [73.9–87.7]	0.21		
End of enrolment	≤2005	6	85.0 [76.3–93.8]	1.00		
	2006–2009	20	85.0 [80.3–89.7]	Ref		
	≥2010	6	78.3 [67.8–88.9]	0.26		
Region	Western Africa	8	85.0 [78.1–91.9]	0.64		
	Eastern Africa	8	89.4 [84.9–93.9]	Ref		
	Southern Africa	11	81.0 [75.3–86.6]	0.11		
	Central Africa	4	83.2 [77.2–89.1]	0.51		
	Several region	1	62.3 [60.3–74.3]	0.010		
Type of site	Public sector	28	85.4 [81.6–89.3]	Ref	Ref	Ref
	Other	3	68.2 [60.9–75.5]	0.005	0.030	0.008
Number of sites	1	18	83.9 [78.0–89.8]	Ref		
	≥2	8	82.3 [77.2–87.3]	0.70		
Decentralized area	No	24	82.5 [78.1–86.9]	Ref		
	Yes	7	88.5 [83.7–93.3]	0.21		
Population selected	General	24	84.1 [79.8–88.4]	Ref		
	General, with FU >6 months	5	77.8 [67.5–88.1]	0.14		
VL monitoring before evaluation	No	11	82.6 [73.2–92.0]	Ref		
	Yes	21	84.3 [80.2–88.3]	0.67		
Tracking of LTFU patients	No	24	82.2 [77.4–86.9]	Ref		
	Yes	8	88.2 [82.7–93.8]	0.13		
Median age at ART initiation (years)	≤36	13	87.7 [83.3–92.2]	Ref	Ref	
	>36	15	80.3 [73.7–87.0]	0.044	0.18	
Proportion of women	≤65	12	79.1 [72.1–86.1]	Ref	Ref	Ref
	>65	20	86.5 [83.4–89.7]	0.020	0.29	0.09
Proportion of patients in WHO stage 3–4	≤69	11	83.3 [76.8–89.9]	Ref		
	>69	11	86.7 [82.2–91.2]	0.45		
Median CD4 at ART initiation (cells/mm^3)	≤135	18	82.6 [78.1–87.1]	Ref		
	>135	12	85.8 [77.5–94.0]	0.41		
Median VL at ART initiation (log copies/mL)	≤5.2	5	91.7 [87.8–95.7]	Ref	Ref	
	>5.2	8	83.0 [75.5–90.5]	0.09	0.24	
Proportion of EFV exposed patients	≤40	13	82.5 [74.7–90.4]	Ref		
	>40	10	82.6 [75.6–89.7]	0.98		
Proportion of NVP exposed patients	≤60	13	79.7 [73.0–86.5]	Ref		
	>60	10	86.4 [81.3–91.6]	0.12		

https://doi.org/10.1371/journal.pone.0174767.t003

Using an ITT approach, 5 evaluations of virological success at 36 months after ART initiation were available at the threshold of 400 copies/mL, and 2 evaluations provided proportions of virological success at the thresholds of 1000 copies/mL. The overall proportions (95% CI) of virological success at these thresholds were 37.8% (22.7–52.9) and 31.6% (0.03–59.8) (S1 Fig),

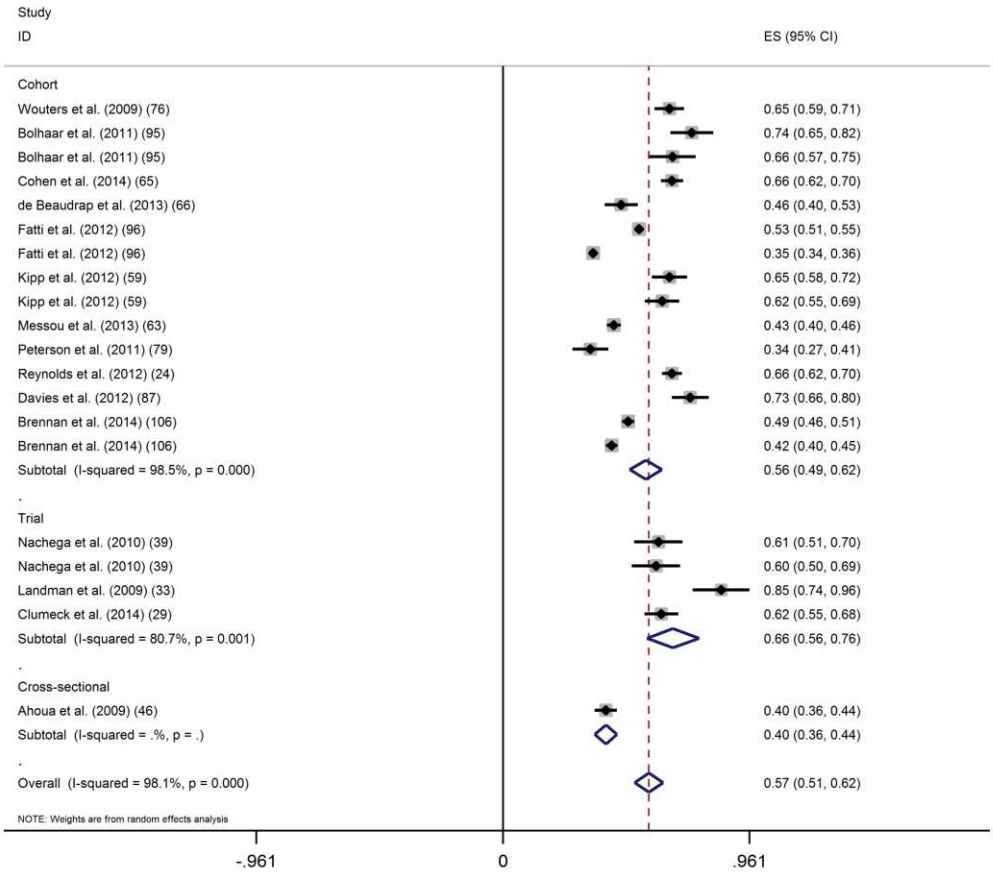

Fig 5. Intention-to-treat analysis of the proportion of virological success (400 copies/mL) at 24 months of ART by study design.

https://doi.org/10.1371/journal.pone.0174767.g005

respectively. Only one evaluation was performed at the threshold of 5000 copies/mL. This indicated a 63.3% proportion of virological success (95% CI: 59.3–67.3) [24] (S1 Fig).

Evaluation of publication bias. The funnel plots of the individual studies showed an asymmetry, with smaller studies reporting higher proportions of virological success more often (S2 Fig). Applying the method proposed by Eger and colleagues, the presence of publication bias was evidenced in the ITT analysis at 24 months (p-value for the intercept: 0.018), but not in any other analysis.

Discussion

In this meta-analysis, we reported the proportion of patients in virological success while on first-line ART in sub-Saharan Africa, based on 85 recently published articles that led to 125

Table 4. Rate of virological success (<400 copies/mL) at 24 months–intention-to-treat analysis.

				Univariate analysis	Multivariate analysis	
Variable	**categories**	**N**	**Rate (95% CI)**	**(p)**	**Full model (p)**	**Reduced model (p)**
Study design	Cohort	16	54.6 [48.6–60.6]	Ref	Ref	
	Trial	4	66.2 [56.1–76.4]	0.14	0.88	
Beginning of enrolment	≤2005	14	58.5 [50.2–66.7]	Ref		
	≥2006	6	53.0 [46.9–59.2]	0.52		
End of enrolment	≤2005	4	58.7 [41.9–75.6]	0.92		
	2006–2009	12	60.7 [53.0–68.3]	Ref		
	≥2010	4	44.8 [35.4–54.3]	0.24		
Region	Western Africa	4	51.2 [37.6–64.8]	0.84		
	Eastern Africa	4	58.1 [44.3–72.0]	Ref		
	Southern Africa	11	58.0 [50.5–65.5]	0.99		
	Central Africa	1	61.7 [55.1–68.3]	0.99		
	Several region	-	-	-		
Type of site	Public sector	17	59.4 [53.7–65.2]	Ref	Ref	Ref
	Other	3	42.8 [29.4–56.2]	0.05	0.43	0.29
Number of sites	1	14	59.7 [52.9–66.5]	Ref		
	≥2	3	50.1 [39.3–60.8]	0.32		
Decentralized area	No	14	57.1 [50.9–63.4]	Ref		
	Yes	3	62.4 [57.6–67.2]	0.86		
	Both	2	44.1 [26.5–61.5]	0.39		
Population selected	General	15	55.3 [48.9–61.7]	Ref		
	General, with FU >6 months	3	51.2 [37.9–64.6]	0.62		
	Specific	-	-	-		
VL monitoring before evaluation	No	4	48.0 [35.8–60.3]	Ref		
	Yes	16	59.1 [53.2–65.0]	0.15		
Tracking of LTFU patients	No	13	61.6 [54.8–68.5]	Ref	Ref	Ref
	Yes	7	48.2 [39.6–56.8]	0.048	0.46	0.22
Median age at ART initiation	≤36 years	9	54.6 [45.4–63.8]	Ref		
	>36 years	9	56.1 [49.4–62.9]	0.78		

https://doi.org/10.1371/journal.pone.0174767.t004

independent study populations comprised of 156,798 individuals. To our knowledge, this is the most recent and largest meta-analysis to compare virological success at specific time points in sub-Saharan Africa.

We did not observe an improvement in the proportion of virological success as compared to previous meta-analyses [8–10, 15, 17]. Our meta-analysis however is novel in the way in which we restricted our search to studies implemented in sub-Saharan Africa, and for the fact that it is not largely based on clinical trials. As compared to previously published meta-analyses which were not restricted by geographic location, and largely based on the results of clinical trials [15, 17], we believe that our meta-analysis is more representative of real-life treatment and care settings in sub-Saharan Africa–the region with the highest HIV burden.

In all the studies considered in this meta-analysis, the most common threshold for the evaluation of virological success was 400 copies/mL. Using this threshold and an OT approach, (e.g. only considering those still in care and on ART at the time of evaluation), the proportion of virological success was around 85%. Interestingly, this proportion of virological success remained stable regardless of the time point between M6 and M36. This result is likely due to

the fact that only those who remain alive and in care are considered, which induces a healthy survivor bias–a bias which has been reported previously [17].

Several of the studies included in the meta-analysis also reported virological success at the threshold of 1000 copies/mL. This is the threshold for virological failure as defined by the latest WHO recommendations [110]. Using an OT approach, increasing the threshold from 400 to 1000 copies/mL did not increase the proportion of virological success by a significant amount, suggesting that few patients exhibit intermediate replicative levels. From a virological point of view, defining success at the threshold of 400 or 1000 copies/mL raises the potential for acquisition of drug resistance at a low level of replication [111, 112]. From a public health perspective, our results suggest that defining virological success at the threshold of 1000 or 5000 copies/mL is not identical, which is in accordance with the 2013 WHO recommendations in which the threshold defining virological success was lowered from 5000 to 1000 copies/mL [4]. Our results indicate that broadening access to viral load monitoring for patients under ART, especially through use of tools such as dried blood spots (which allows for a threshold of 1000 copies/mL, but not any lower) [113–116] should be more of a priority than lowering the threshold defining virological success. These findings support the WHO guidelines, in which virological failure remains at the threshold of 1000 copies/mL.

The results of our meta-analysis on the proportions of virological success at 6, 12, 24 and 36 months after ART initiation defined at the thresholds of 400 and 1000 copies/mL are comparable to the results from another meta-analysis performed in resource limited settings [17], despite this meta-analysis aggregating studies irrespective of the threshold.

Of the 125 study populations considered, only 28 (22.4%) directly reported the proportion of patients in virological success using an ITT approach. To better estimate the proportions of success, we used results when reported using an ITT approach, but also searched the articles for the number of deaths, loss to follow-up, switches to 2nd line ART, transfers/withdrawals, and for the number of patients who did not have a virological evaluation. This enabled us to estimate the proportion of patients in virological success using an ITT approach in an additional 38 studies.

Regardless of the time point following ART initiation and the threshold used to define virological success, results using an ITT approach were lower than those using an OT approach. OT results evaluate ART efficacy for those still on ART, while ITT results evaluate the effectiveness of the programmes delivering ART, as those who died or were lost to follow-up are considered as failures, and these numbers increase with time. The difference in estimated proportions between the two approaches was substantial and as large as 15% at 6 months after ART initiation, reaching 20% at 12 months, and 25% at 24 months. This difference can be explained by death and patients LTFU, but also by the number of patients who did not have a virological evaluation performed. Studies in which the proportion of non-evaluated patients was >40% [96, 99, 100, 117, 118] were not considered in the ITT analysis, as we sought "systematic" virological evaluation, and it is possible that a lack of viral load monitoring was not random.

We wanted to investigate whether clinical trials, cohort studies, and cross-sectional studies provide similar quality information on proportions of virological success.

The fact that the proportions of virological success, using an OT approach, compare well across the various study designs indicates the effectiveness of ART in Africa. It also seems to indicate that patients in routine care are as adherent to ART as those enrolled in trials, even though the latter group may be more sensitised to this issue.

Cross-sectional studies, if based on a representative sample of the targeted population, have fewer logistical and financial constraints, allowing results to be obtained more quickly than cohort studies. Such studies are of special interest for national HIV treatment programmes, as

they provide valuable data for the planning of future activities. They are also recommended by WHO for the surveillance of resistance in patients on ART [119].

On the other hand, using an ITT approach, the proportion of virological success was significantly higher in randomised trials than in cohorts at 12 months after ART initiation, and tended to be higher at 24 months. This could be explained by the selection of healthier participants, but also by more frequent monitoring of patients, more systematic clinical and biological examinations and by reinforced clinical teams in trials as compared to routine practice. All of these factors may have reduced attrition in clinical trials, and may explain the higher proportion of virological success as compared to cohort studies. Indeed, a relatively high mortality in the first 6 to 12 months after ART initiation and substantial loss to follow-up have been widely reported in resource limited settings [22, 23, 120, 121]. These results highlight the need to strengthen retention in care in these settings.

Unexpectedly, in ITT analysis there was no evidence of an effect of tracking of LTFU patients on the proportions of virological success at 12 months after ART initiation, and at 24 months the proportion of virological success was even lower in studies that tracked LTFU patients than in studies that did not. However, this result is likely influenced by two studies that implemented tracking, but which had very low proportions of virological success. In one of these studies, there was a very large (32%) proportion of deaths [79]. The other study was a multicentre nation-wide study in South Africa that reported particularly low ITT results [96]. This likely illustrates the heterogeneity in the definition of tracking of LTFU patients which ranges from limited telephone contact efforts to more intensive home visits. Despite this meta-analysis not finding a strong link between the tracking of LTFU patients and virological success, such initiatives should be more widely implemented as a means to maintain patients in care and to prevent the acquisition of resistance.

The proportion of patients in virological success in OT analysis was found to be larger in public health centres compared to other sites (faith-based, NGO supported or with a mixed recruitment). Public centres may have been selected based on their capacities to participate in such studies (e.g. sufficient number of medical staff, easy access to a biological laboratory), but these are not necessarily representative of all health centres in the country. Faith-based and NGO supported centres may treat lower socio-economic populations and data from these centres may be under-reported, leading to a publication bias. Motivations to publish may differ between centres, while the funnel plots used in this meta-analysis indicated that some small study bias may be present.

Some limitations of this meta-analysis should be noted. Studies considered were heterogeneous in their design but also in the populations they considered. Most studies were based on the general population with limited selection criteria, but some were restricted to specific populations, such as patients co-infected with hepatitis or tuberculosis, or women who had previously been exposed to ART for PMTCT, etc. Studies on these specific populations had a limited sample size, and were therefore thought to contribute to the heterogeneity among estimations. However, the effect of these specific populations was investigated and was not found to be significant.

The studies also differed in terms of their definition of virological success, partly due to the variability of the techniques used to measure viral load, which do not all have the same thresholds. To control for this variability, we performed estimations of virological success at various thresholds. In only a small number of studies was virological success based on the result of two viral load measurements. This may also have contributed to the heterogeneity of our results. However, the limited number of such studies meant that this effect on the meta-analysis could not be evaluated.

To explain the heterogeneity of virological success across various study populations, programme characteristics were investigated (e.g. tracking of LTFU patients, type of site, setting). However, no specific characteristics were identified. This may be explained by characteristics not being mentioned in the individual studies, resulting in the pooling of studies in the meta-analysis that were not in fact similar.

By definition, only studies with virological evaluation were considered in our meta-analysis. Many programs in Africa, where no viral load monitoring is available have thus not been considered in our meta-analysis. However, we do not anticipate that this is a source of bias in our results. Indeed, some of the studies we considered were virological evaluations at a specific time point, without routine viral load monitoring. This was particularly true for cross-sectional evaluations, nested in cohort studies, and we did not find evidence that results from cross-sectional evaluations differed from the other studies.

In conclusion, this is the largest, most up-to-date meta-analysis on proportions of virological success at specific time points after ART initiation and at multiple thresholds, based on both OT and ITT analysis and the first comparison between clinical trials, cohort studies, and cross-sectional studies. Evidence from this review suggests that the current international target to have 90% of patients on ART in virological success will require intensive strategies to improve adherence to ART, and retention in care. It is also important to improve access to viral load monitoring in order to detect those at risk of virological failure, as early as possible, offering them effective ART.

Supporting information

S1 Checklist. PRISMA 2009 checklist.
(DOCX)

S1 Fig. Estimation of the rate of virological success based on different threshold (in copies/mL) on on-treatment (full box) and intention-to treat (empty box) at: A) at 6 months of ART, B) 12 months of ART, c) 24 months of ART, and D) 36 months of ART.
(DOCX)

S2 Fig. Funnel plots of the rate of virological success (defined at the threshold of 400 copies/mL) against sample size. A) OT analysis at 12 months; B) ITT analysis at 12 months; C) OT analysis at 24 months; D) ITT analysis at 24 months.
(DOCX)

S1 Table. Quality evaluation of the articles based on the STROBE statement.
(DOCX)

Acknowledgments

We would to thank Ms Sandrine Royer-Devaux for her support in the article search and Ms Rebecca Grant and Ms Anna Louise Funk for their thorough proof reading and editing.

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Writing – original draft: FT YM.

Writing – review & editing: FT YM AC ED.

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Dans le cadre de l'évaluation de l'efficacité des TARV, les études transversales se révèlent aussi performantes que les études de cohortes. Ces dernières présentent moins de contraintes logistiques, moins d'investissements financiers et permettent de générer des résultats plus rapidement que les études de cohortes ou les essais cliniques. Ainsi, pour les premières applications de l'étude du contrôle virologique en utilisant le support de prélèvement par DBS au Cameroun, nous avons mené une étude transversale.

Dans le cadre de l'évaluation de l'efficacité d'un programme de santé, la méta-analyse réalisée a montré une différence entre les résultats de contrôle virologique issus des essais et ceux issus des cohortes. Cette différence est essentiellement liée au taux moindre de décès et de perdus de vue ainsi qu'à une meilleure adhérence dans les essais. Dans le cadre d'un projet de recherche opérationnelle comme le nôtre, ayant pour objectif d'apporter des données de terrain collectées en « condition de vie réelle » et en vue d'une extrapolation optimale des résultats générés, nous avons mis en place, au Vietnam, une cohorte de patients infectés par le VIH inclus au moment de l'initiation de leur TARV. De plus, il est intéressant de noter l'absence de différence significative entre les résultats de succès virologiques définis par un seuil à 400 cp/mL et à 1000 cp/mL. Ainsi, le seuil de 1000 cp/mL validé par l'OMS dans ces dernières recommandations et utilisé dans le cadre de mesure de la CV sur DBS, a été utilisé dans nos études.

Dans les études que nous avons menées, nous avons apporté une attention toute particulière à la collecte des données de rétention en les collectant dans le cadre de la cohorte et en reconstituant une cohorte dans le cadre de l'étude transversale via une collecte de données rétrospective. Ainsi, nous avons pu rapporter une analyse en « intention-de-traiter » pour l'ensemble de ces études, apportant des informations précieuses aux programmes de santé et minimisant ainsi le biais d'attrition.

Les premières études présentées sont celles menées au Cameroun.

Levels of early warning indicators in decentralized HIV care sites in Cameroon. Article en cours de finalisation

Levels of early warning indicators in decentralized HIV care sites in Cameroon

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Abstract

Background Although access to antiretroviral therapy (ART) has drastically increased in the last years, access to viral load monitoring is still scarce. Therapeutic failure is thus difficult to diagnose and patients are maintained on sub-optimal ART, increasing the risk to develop and transmit HIV drug resistances. Early warning indicators (EWIs) allow to estimate this risk at site level.

Methods Retrospective cohort study of all HIV-1 infected adults who initiated ART between June and February 2015, in 12 sites in 3 regions in Cameroon. All participating sites were decentralized primary care sites. At site level, three EWIs were estimated. Patients who were still in care 12 months after ART initiation were invited to be virologicaly evaluated.

Results A total of 771 patients initiated ART in the 12 HIV care sites during the study period (range: 22-141). In all sites, prescribing practice fulfilled national and WHO guidelines. On the other hand, the rate of loss to follow-up and hence the retention rate, the proportion of adherent patients, and proportion of virologicaly suppressed patients did not reach the WHO standards in most sites.

Conclusion EWIs documenting rates of retention, virological suppression and adherence to ART were alarmingly low in several sites. The values of these three indicators were coherent, and call for a better understanding of the difficulties met by the patients. Initiatives to improve HIV care are urgently needed.

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45 # Introduction

46 Despite important advances, HIV remains a major health issue worldwide, and particularly in sub-
47 Saharan Africa where the majority of patients live [1]. From 2010 to 2015, the number of HIV-infected
48 individuals receiving antiretroviral therapy (ART) in sub-Saharan Africa increased from 5 million to more
49 than 15 million [2] . As of today, routine viral load (VL) monitoring is still scarce in Africa [3, 4] although
50 it is well known that immuno-clinical criteria perform poorly to identify patients in therapeutic failure
51 [5-7]. Thus, those failing are maintained under the same sub-optimal ART regimen, what contributes to
52 the increase of both transmitted and acquired HIV drug resistances (HIVDR) [8-10].

53 The World Health Organization (WHO) developed Early warning indicators (EWIs), estimated at site
54 level, to help monitor ART programs and identify aspects that are not reaching acceptable levels of
55 performance and which may trigger emergence of HIVDR [8, 11, 12]. These EWIs monitor ART supply,
56 ART delivery, retention in care, adherence to ART and virological suppression in line with the UNAIDS
57 90-90-90 goals [11-13]. The EWIs can be calculated with routine healthcare information and adapted to
58 national resources, are useful to guide initiatives to be taken to improve HIV care and prevent
59 emergence of HIV DR.

60 In Cameroon, HIV care and ART are provided free of charge since 2007 in HIV care sites throughout the
61 country as part of a decentralization strategy. Recently, ART eligibility was widened to patients
62 regardless of CD4 count [14]. Routine VL monitoring is still not available nationwide, VL measurement is
63 at the expense of the patients and only available in a limited number of laboratories in large cities and
64 hence scarcely used. In Cameroon, EWIs have been previously estimated [15-17]. However none of these
65 reports evaluated the proportion of virologicaly suppressed patients (VL ≤1000 copies/mL). Moreover,
66 one report showed a dramatic deterioration of these EWIs [15]. The goal of this study was to evaluate
67 the EWIs to monitor HIVDR, in primary decentralized HIV care sites in Cameroon, where no routine VL
68 monitoring is implemented.

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Materials and Methods

Study design

The study was conducted in 12 HIV care sites randomly selected from a list of 70 eligible HIV care sites established with the help of the National Committee of Fight against AIDS (CNLS). The following selection criteria were considered: i) in 3 regions of Cameroon (Centre, East, and North-West) to cover a wide part of the country; ii) in decentralized areas and iii) with an active number of patients on ART between 100 and 500 to limit heterogeneity due to site capacity.

From September 2015 to January 2016, a cross-sectional evaluation was conducted to assess virological status at 12 months of ART in patients on 1st line ART in the selected sites. Patients were eligible if they were: HIV-1 positive, had initiated ART between June 2014 and February 2015 (so that patients were on ART for 11 to 15 months at the time of virological evaluation), and were 21 years of age or more at ART initiation. At the clinical visit, clinical data were recorded in a standardized case report forms and adherence to ART was assessed by the self-reported number of missed doses during the previous four days [18]. Those reporting no missed-dose were classified 100% adherent while those reporting at least one missed dose were classified as not 100% adherent. Then a retrospective cohort was constituted from the medical charts of the selected sites. Individual data on all HIV-1 mono-infected adults who had initiated ART during the study period, the following data were extracted from the clinical charts using a standardized case report form: HIV-1 serology, date of ART initiation, ART regimen prescribed since initiation along with dates and reasons of modification, number of clinical visits respected since ART initiation, vital status and date of vital status evaluation.

Additionally, health care personnel was interviewed to record data about the HIV care site and in particular, the experience in HIV care, workload (estimated as the ratio between the number of patients and the number of health care staff), supervision and trainings received, pharmacy stock-outs in the previous 12 months, technological capacity, and costs for the patients.

All analyses were performed using Stata 13 (College Station, Texas, USA).

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Early Warning Indicators

Three of the five EWIs defined after the 2011 review (Table 1) [19] were estimated at the site level: Dispensing practices (EWI4), retention in care (EWI2), and virological suppression at the threshold of 1000 copies/mL (EWI5). For EWI5, patients who refused to participate to the study and patients to whom the study was not be proposed were excluded, on the other hand patients who had sent a third party to collect their ART were considered in virological failure.

Table 1. Definition and calculation rules of the three early warning indicators (EWIs) defined after the 2011 revision

EWI	Definition	Numerator	Denominator	Target
EWI2 – Retention in care	percentage of adults known to be alive and on treatment 12 months after initiation of ART	Number of adults who are still alive and on ART 12 months after initiating treatment	Total number of adults who initiated ART who were expected to achieve 12-month outcomes within the reporting period, including those who have died since starting therapy, those who have stopped therapy, and those recorded as lost to follow-up at month 12	• Red <75% • Amber, 75-85% • Green >85%
EWI4 – Dispensing practices	percentage of adults prescribed or picking up mono or dual ARV therapy	Number of patients who pick-up from the pharmacy, a regimen consisting of one or two ARVs	Number of patients picking up ART on or after the designated EWI sample start date.	0%
EWI5 – Virological suppression	Percentage of patients receiving ART at the site after the first 12 months of ART whose viral load is <1000 copies/ml	Number of patients receiving ART at the site after the first 12 months of ART whose viral load is <1000 copies/ml	Number of patients at the site who by national policy should have had a viral load performed 12 months after ART initiation	• Red <75% • Amber, 75-85% • Green >85%

EWI: early warning indicator

Former EWIs (2008 definition) [19] were also estimated to allow comparison with results previously published. The six EWIs-2008 (S1 Table) monitored: prescribing practice (EWI-2008-1), loss to follow-up 12 months after ART initiation (EWI-2008-2), maintenance on appropriate first-line ART at 12 months

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108 (EWI-2008-3a), ART drug supply continuity (EWI-2008-6c1), self-reported adherence to ART (EWI-2008-
109 7), and viral load suppression 12 months (EWI-2008-8).

110 Each HIV care site was then classified as reaching or not the target defined by the WHO for each of these
111 EWIs and EWIs-2008.

112 Viral load quantification

113 During the 12 months follow-up visit at the HIV care site, a 5 ml blood sample was drawn and 5 whole
114 blood spots of 50µl were deposited on each of two Whatman 903 filter paper cards. The dried blood
115 spot (DBS) cards were left to dry for 3 hours at ambient temperature before being individually packed
116 in sealed bags with two desiccants, and stored in a dedicated box at ambient temperature. The DBS
117 cards were transferred weekly to the IRD-CREMER virology laboratory in Yaoundé (Cameroon) for viral
118 load measurement.

119 At the IRD-CREMER laboratory, following the manufacturer's protocol, two spots of 50µl were cut and
120 placed in 1.7mL of lysis buffer provided in the Abbott m2000sp preparation kit, and was left for 30mn
121 under constant agitation. Then, 1ml of the eluate was placed in a tube and centrifuged for 10mn at
122 2000rpm in order to remove small paper particles from the supernatant, which is then used like plasma
123 for RNA extraction and measure of the viral load using the m2000rt Abbott real-time PCR.

124 Ethical clearance

125 The protocol of the study was submitted in Cameroon to the National Ethics Committee of research for
126 human health (CNERSH, ethical clearance number 2015/04/580/EC/CNERSH/SP), and in France to the
127 Institutional Review Board (IRB) of Institut Pasteur in Paris (France).

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129 Results

130 Description of the HIV care sites

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131 The 12 primary HIV care sites considered were distributed in three regions of Cameroon, far from large
132 urban centers. Of these HIV care sites, 7 (58%) were public, 4 (33%) were classified as private
133 confessional institutions and the remaining one as private but non-confessional. All HIV care sites had
134 experience in providing HIV care, their experience ranging from 5 to 12 years. The median (inter-quartile
135 range (IQR)) number of HIV patients followed in each care site at the time of the evaluation was 593
136 (242-1055), and of care staff was 8 (4.5-10.5). The median (IQR) number of patients per care staff was
137 109 (53-168). Second-line ART was available in 4 (33.3%) care sites; in the other sites patients were
138 referred to a care site of higher level to obtain second-line ART. Only 6 (50%) care site were able to
139 locally measure CD4 cell counts had CD4 testing machines (PIMA Alere™ or Cyflow™). No care sites were
140 able to perform VL measurements. All sites declared that active tracking procedures were implemented
141 in case of loss to follow-up, firstly by phone and secondly by mobilization of community agents if the
142 first attempt was unsuccessful. All but one sites declared that a consultation dedicated to adherence
143 reinforcement was organized. The median distance to the furthest location served by the HIV care site
144 was 135 Km (range: 35 and 300 Km).

145 Between June and December 2014, a total of 771 HIV-1 infected patients initiated ART in the 12 HIV care
146 sites (Table 2). This number widely varied across sites (range: 22-141). Of these patients, a total of 218
147 became lost-to follow-up and 65 died.

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159 Table 2: Patient's characteristics (retrospective cohort)

		N=771
HIV care sites, n (%)		
Central region		186 (24.1)
	A	78 (10.1)
	B	22 (2.8)
	C	47 (6.1)
	D	39 (5.1)
Eastern region		255 (33.1)
	E	63 (8.2)
	F	47 (6.1)
	G	35 (4.5)
	H	110 (14.3)
North-West region		330 (42.8)
	I	56 (7.3)
	J	68 (8.8)
	K	65 (8.4)
	L	141 (18.3)
Male gender, n (%)		223 (28.9)
Age at ART initiation (years), median (IQR)		39 (32 – 46)
Initial ART, n (%)		
	3TC+TDF+EFV	511 (66.3)
	3TC+TDF+NVP	59 (7.6)
	3TC+AZT+EFV	53 (6.9)
	3TC+AZT+NVP	132 (17.1)
	Other	16 (2.1)
CD4 level at ART initiation (cells/mm^3),		
	N (%)	501 (65.0)
	median (IQR)	185 (79 – 316)

160 ART: antiretroviral therapy; IQR: inter quartile range

161

162 Levels of EWIs

163 EWI2 monitored the retention in care (Table 3). No sites achieved the target of more than 85% of
164 retention rate. Only 3 (25%) sites achieved the target of more than 75% of retention rate. Among the
165 remaining 9 HIV care sites, 6 presented a retention rate above 50% and 3 below this threshold. In the
166 previous definition of EWIs, two EWIs monitored two different aspects of retention. EWI-2008-2
167 monitored the percentage of patients lost to follow-up (LTFU), and again only 3 (25%) sites showed less
168 than 20% of LTFU. EWI-2008-3a monitored the percentage of patients still on first-line ART at 12 months,
169 the same 3 (25%) sites were the only reaching the target of >70% (supplementary table 2) however this
170 low level was essentially due to the deaths and the high rate of LTFU. Indeed, among patients still in care
171 at 12 months, only 4 patients were receiving a protease-inhibitor (PI)-based second-line ART. Moreover,
172 EWI2 and EWI-2008-3a were strongly correlated (spearman ρ=-0.93, p<0.001).

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173 Table 3: Estimation of the three early warning indicators (EWIs)

HIV care site	EW2	EWI4	EWI5
	Retention in care	Dispensing practices	Virological suppression
Centre	**n=186**	**n=186**	**n=109**
A	67.9%	**0%**	83.3% (n=48)
B	54.6%	**0%**	85.7% (n=7)
C	63.8%	2.1%*	60.7% (n=28)
D	76.9%	**0%**	53.9% (n=26)
East	**n=255**	**n=255**	**n=121**
E	79.4%	4.8%**	41.5% (n=41)
F	38.3%	**0%**	50.0% (n=18)
G	42.9%	**0%**	71.4% (n=14)
H	48.2%	**0%**	52.1% (n=48)
Northwest	**n=330**	**n=330**	**n=215**
I	62.5%	**0%**	61.7% (n=34)
J	77.9%	1.5%*	59.6% (n= 52)
K	61.5%	**0%**	80.0% (n=40)
L	70.2%	2.1%**	44.9% (n=89)

174 * 1 patient with initial ART not specified in the medical file ** 3 patients with initial ART not specified in
175 the medical file

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177 In 8 (67%) HIV care sites, no patient was initiated on a mono- or dual-drug regimen (EWI4). In the
178 remaining 4 sites, EWI4 did not reach the target of 0% because the initial ART that was not notified in
179 the medical file, this concerned from 1 to 3 patients in these sites (Table 3). The previous EWI related to
180 the dispensation of a NNRTI-based first-line regimen (EWI-2008-1). This EWI was similar in the 4 sites
181 where the initial ART was unspecified, and in 2 other sites some patients were initiated on a PI-based
182 regimen (S2 Table).

183 The current EWI monitoring virological suppression (EWI5) is defined using an on-treatment definition.
184 Thus, out of the 771 patients who initiated ART, 445 patients who were virologicaly evaluated were
185 considered. The remaining patients were lost to follow-up (n=218), dead (n=65), refused to participate
186 (n=3) or have not be invited to participate in the study (n=40). Only one care site achieved a percentage
187 of patients with viral load <1000 copies/mL higher than 85% and this site only evaluated 7 patients (Table
188 3). In the remaining sites, the percentage of patients with virological suppression ranged between 75%
189 and 85% in 3, and was below 75% in the remaining 8 (66%). The previous EWI dealing with virological
190 suppression (EWI-2008-8) was defined using an intention-to-treat definition, thus considering death and

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191 LTFU patients as virological failures. The rate of virological suppression was thus even lower, and none
192 of the sites achieved the target of 70%.

193 In the previous definition of EWI, EWI-2008-7 was related to adherence to ART using a standardized
194 measure. It was evaluated, only in those who participated to the cross sectional evaluation, through self-
195 report of the number of missed dose during the last 4 days. Overall, none of the HIV care sites considered
196 reached the target of 90% of the patients being fully adherent. In three sites, the proportion of fully
197 adherent patients was close to the target (range: 80.9%-85.0%), while in the remaining ones the
198 proportion ranged from as low as 14.3% to 73.3% (supplementary table 2).

199 Not having access to pharmacy data, EWI3 that monitors pharmacy stock-outs could not be estimated.
200 However, we estimated the EWI-2008-6c1 which monitors the number of patients who were still
201 followed and for whom ART was interrupted or changed due to drug stock-out. Half of the HIV care sites
202 had achieved the target of no ART interruption or change due to stock-outs. In the remaining 6 sites, the
203 1 to 4 patients had interrupted or changed ART due to drug stock-out, corresponding to proportions
204 ranging from 0.9% to 5.9% of all patients who had initiated ART during the study period.

205

206 Discussion

207 In recent years, many efforts have led to a wider access to ART in many low- and middle-income
208 countries. The need to monitor HIV programs, especially in regard to the risk of emergence of HIVDR
209 acquisition and transmission, motivated the WHO to define standardized tools to monitor the risk of HIV
210 DR [11]. This is of particular importance in a context where routine VL monitoring is still scarce.

211 We evaluated three of the current EWIs and six former EWIs (i.e. 2008 definition) in 12 decentralized
212 rural primary HIV care sites. These sites were of variable size and capacity, but represent the standard
213 of care for an important proportion of patients in Cameroon. As of today, these care sites, far from large

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214 urban centers, do not offer routine VL monitoring and HIV care still relies on clinical and immunological
215 monitoring, when CD4 testing is available.

216 Within the first 12 months following ART initiation, in 4 of the 12 sites investigated, about half of the
217 patients were already LTFU (EWI2 ranged from 42.9% to 59.1%). Three of these sites were in the East
218 province and were large sites as they reported currently following between 214 and 1,112 HIV patients.
219 The remaining site was from the central region and of modest size with a declared total of 85 patients
220 currently followed. On the other hand, 3 care sites achieved the target of <20% of loss to follow-up; it
221 was even as low as 3.6% in one site where 436 HIV patients were currently followed. This latter result
222 indicating that the workload at the site level is not the sole reason that could explain the high level of
223 loss to follow-up. All the investigated sites declared that active tracking of LTFU was implemented, but
224 measures and human resources might vary between sites. Other studies have pointed out the alarmingly
225 high rate of LTFU in Cameroon [15, 16, 20]. In depth analysis of the various measures implemented to
226 prevent LTFU should be conducted through a multidisciplinary approach to better understand why the
227 result is unsatisfactory in some sites, and to identify correcting measures to improve this alarming
228 situation.

229 EWI4 monitors dispensing practices of 1st line ART, and more precisely is used to make sure that no
230 patients are receiving a mono- or dual-molecules ART regimen as per national and WHO guidelines. This
231 EWI was highly respected. Only in few sites was the EWI4 not 0%, but the reason was exclusively because
232 the initial ART combination was not reported in the medical charts at the site level. Although this
233 concerned only 1 to 3 patients per care site, it is a negative point as these medical charts are the HIV
234 history of the patients and such information is crucial for good HIV care to maintain prescription of the
235 same first line and when a change of line will be required. In all sites, patients initiated an NNRTI-based
236 first-line ART. This first-line ART was nearly exclusively (98.9%) 3TC-TDF or 3TC-AZT associated either to
237 EFV or NVP, reflecting the WHO and national guidelines, but also reflecting the limited number of
238 molecules currently accessible in Africa. This also may explain that modification or interruption of ART

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239 due to drug stock-outs was rare in the sites investigated. Per site, the number of patient concerned
240 ranged from 0 (in 50% of the sites) to 4. Of note, only 4 patients were switched to a PI-based second-
241 line ART. Although the number of patients on second-line ART is expected to be low at 12 months of
242 ART, this extremely low number probably illustrates the difficulties to diagnose and possibly to deal with
243 failure. It may also be explained by the fact that not all sites can provide second-line ART. Among the 12
244 sites considered, only 4 were able to deliver second-line ART, in the other sites the patients had to
245 transfer to another clinical care site.

246 The essential EWI might however be the one estimating the proportion of virologicaly suppressed
247 patients (EWI5), defined by a VL ≤1000 copies/mL 12 months after ART initiation and in line with the
248 third goal of the 90-90-90 UNAIDS Treatment Targets. The current definition of this EWI considers all
249 the patients who, by national policy, should have had a VL performed 12 months after ART initiation.
250 This definition excludes patients who are already LTFU or dead, and is therefore using an on-treatment
251 approach. All the care sites investigated showed a low proportion of virologicaly suppressed patients,
252 the overall proportion was 67.3%, much lower than the target of >80%. This rate was however in line
253 with previous evaluations [21, 22]. These 4 sites also presented the worst virological suppression rates,
254 whether using an on-treatment (EWI5) or an intention-to-treat (EWI-2008-8) definition. Moreover, the
255 number of patient with a low viral replication (between 200 and 1000 copies/mL) was not considered in
256 the WHO criteria and represent an additional risk of HIV DR acquisition [23, 24].

257 Viral load measurements were performed on DBS using the Abbott protocol. The latest WHO guidelines
258 mention that DBS can be used in settings where plasma is not feasible [25]. Moreover, several studies
259 have evaluated the specificity of this technique to diagnose virological failure at the threshold of 1000
260 copies/mL, and it was found to be around 95% [26-30]. Finally, all VL measurement from this study have
261 been performed in the same laboratory that has a very strong expertise both on plasma and DBS [27].
262 Therefore, DBS use can be ruled out as the reason why the proportion of virologicaly suppressed patients
263 was so low.

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264 When an intention to treat approach was performed, i.e. considering LTFU and death as virological
265 failure, as in the former definition of EWI-2008-8, this proportion was even more alarmingly low. It never
266 exceeded 51.3%, and could be as low as 19.1%. This is much lower than what is documented in the
267 literature [21, 22] but consistent with the worryingly high proportion of patients lost to follow-up (EW2).

268 Adherence to ART was self-reported and patients were considered as not fully adherent if they reported
269 having missed one or more ART intake during the last 4 days (EWI-2008-7b). Self-reported adherence
270 has the advantage to be easy to measure, however it has been shown to often over-estimate adherence
271 [31]. We however evidenced a low level of adherence to ART, matching the low level of virological
272 suppression observed in this study, and suggesting that patients not fully adherent correctly reported
273 this behavior. Other studies have also evidenced low level of adherence in Africa [32]. Economic status
274 [33, 34], education level [35, 36] and HIV disclosure [18, 34] have been shown to affect adherence to
275 ART. Still, efforts should be made to understand why patients are not fully adherent. Non-disclosure of
276 HIV-status and stigma are still important barriers to adherence [37, 38]. Lower socioeconomic status and
277 food insecurity are also often associated with lower adherence [37, 39, 40]. Overall, lack of adherence
278 jeopardizes ART efficacy at the individual level, but participates to the HIV epidemics at the population
279 level. It also increases the risk of HIV DR transmission.

280 This study exhibits some weaknesses however. Firstly, the number of patients who initiated ART during
281 the study period in each site, was variable. However, all the patients who initiated ART within the study
282 period were considered in the analysis. Another weakness is linked to its design as the cohort was
283 retrospectively reconstituted based on paper medical charts kept at the clinical site level. If one can
284 expect the information at ART initiation (e.g. date of ART initiation, ART combination received) to be
285 correctly notified in these charts, follow-up data (e.g. date of clinical visit, of drug pick-up) may be more
286 prone to missingness. This could have resulted in over-estimation of loss to follow-up patients in some
287 sites. However, the number of patients still followed according to the charts was consistent with the
288 number of patients virologicaly evaluated. Moreover, patients are assigned a national identification

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289 number in the context of HIV care. No inconsistency was identified such as someone presenting for
290 virological evaluation while being noted as LTFU in the medical charts. Nevertheless, it is possible that
291 some patients who were lost to follow-up re-initiated ART without mentioning this fact and were
292 therefore given a new identification number. Still, these patients probably had an interruption of care
293 and of ART which is detrimental for themselves, and for the population level as well. Considering these
294 patients as lost to follow-up certainly deteriorates the program statistics but raise the alerts regarding
295 compliance to care and to ART.

296 In conclusion, this study adds up evidence of a high risk of HIV DR spreading in Cameroon. Previous
297 results from EWI monitoring warned about the declining adherence rate and frequent disruptions of
298 ART supply in the country. They are now supported by the high proportions of patients in virological
299 failure far from the UNAIDS 90-90-90 treatment targets. Compliance to care needs to be strengthen by
300 national program especially for HIV patients living in decentralized areas. Further molecular studies will
301 be needed to quantify and characterize HIV DR.

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Acknowledgments

We sincerely thank all the patients who agreed to take part in this study, the clinical and laboratory staff from the three HIV care sites who participated in this study: Dr Dongmeza Donald, Ngono Olivier, Dr Kamdom Nicolas, Mrs. Nkodo M. Roanne, Dr Misse Misse P., Dr Ihindi, Dr Ngo Nsoga Marie, Mrs. Ymele Doris, Dr Wandji Kounougo, Mr.Nde Rene Chi, Dr Eugene Chiabi, Mrs. Young Augustine, Dr Bawe Edwin, Mr. Njoya Mamouda, Dr Souore Sanda Jessica, Dr. Essono Mebouinz, Mr. Tize Gabriel, Dr Nyankoue Mebouinz Ferdinand, Zima Mikok Parfait Cyrille, Dr Abeng Brigitte, Mr. Tsague Ngueffo Achille Bosco . We sincerely thanks the surveyors of Care and Health Program organization: Mr. Belinga Henri Louis, Mr. Sinsaï Ronald Nguelewou, Mr. Zamo Alain John, Mr.Matike Ndoumbe René William and Mr. Kamga Eric for his technical support and Mrs. Zobo Atangana Marie Charlotte for her help regarding data entry. We thanks Dr Tsomo Zephirin of Yaounde Central Hospital and Colonel Mpoudi Ngole E. of CREMER. We also are very grateful to the Central Technical Group of the National AIDS Control Committee and the Division of Operational Health Research of the Ministry of Public Health for their support and their help in the implementation of the study. We also thank the Clinical Core from the Center for Translational Research at Institut Pasteur (Paris France) and more specifically Mrs. Cassandre Von Platen for her valuable help in the ethical and regulatory procedures. We thanks Cameroonian office of Expertise France and especially Dr Akamba Jean Jacques. We thanks Expertise France (previously named ESTHER) for their financial support.

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S1 Table. Definition and calculation rules of 6 of the early warning indicators (EWIs) using the 2008 definition

EWI	Definition	Numerator	Denominator	Target
EWI-2008-1	percentage of patients initiating ART at the ART clinic who are initially prescribed an appropriated first-line ART regimen	Number of patients whose medical records explicit initiation of a first-line ART regimen included in the national guidelines	Total number of patients evaluated at the center after 12 months of ART (transferred patients excluded)	100%
EWI-2008-2	Percentage of patients initiating ART at the site who are lost to follow-up 12 months after ART initiation	Number of patients LFTU patients after 12 months of ART	Total number of patients evaluated at the center after 12 months of ART (transferred-out patients and deaths excluded)	<20%
EWI-2008-3a	Percentage of patients initiating ART at the site who are taking an appropriate first-line ART regimen 12 months later	Number of patients treated with appropriated first-line ART who are not dead or LTFU after 12 months of ART	Total number of patients evaluated at the center after 12 months of ART (transferred patients excluded)	>70%
EWI6-2008-c1	Percentage of patients whose regimen was stopped, switched, substituted or incompletely dispensed due to ARV stock-out in a 12 month period	Number of patients whose ART was changed or stopped due to a stock-out	Total number of patients evaluated at the center after 12 months of ART (transferred patients excluded)	0%
EWI-2008-7b	Percentage of patients initiating ART at the site who demonstrate 100% adherence by standardized adherence measure	Number of patients who report having correctly taken their ART in the last 4 days	Total number of patients evaluated at the center after 12 months of ART (transferred patients, dead, LTFU and patients with missing information on this EWI excluded)	>90%
EWI-2008-8	Percentage of patients initiating ART at the site whose viral load is <1000 copies/ml after 12 months of ART	Number of patients still on ART and for whom viral load is <1000 copies/ml after 12 months of ART	Total number of patients who initiated ART (transferred patients excluded)	>70%

EWI: early warning indicator

S2 Table. Estimation of the seven early warning indicators (EWIs)

HIV care site	EWI-2008-1	EWI-2008-2	EWI-2008-3a	EWI-2008-6c1	EWI-2008-7b	EWI-2008-8
	ART prescribing practice	Patients lost to follow-up 12 months after ART initiation*	Patients on appropriate first-line ART 12 months after ART initiation	Patients for whom ART was stopped due to stock-out	Patients reporting 100% adherence to ART**	Viral load suppression 12 months after ART initiation***
Centre	**n=186**	**n=169**	**n=186**	**n=186**	**n=105**	**n=170**
A	**100%**	27.4%	66.7%	**0%**	84.8%	54.8%
B	**100%**	42.9%	54.6%	**0%**	57.1%	35.3%
C	97.9%	26.8%	61.7%	**0%**	60.0%	37.8%
D	**100%**	**11.8%**	**76.9**	2.6%	46.2%	40.0%
East	**n=255**	**n=242**	**n=255**	**n=255**	**n=99**	**n=240**
E	95.2%	**16.7%**	**74.6%**	**0%**	40.0%	31.5%
F	**100%**	59.1%	38.3%	**0%**	58.8%	19.2%
G	**100%**	51.6%	42.9%	**0%**	14.3%	29.4%
H	**100%**	50.5%	48.2%	0.9%	50.0%	23.8%
Northwest	**n=330**	**n=295**	**n=330**	**n=330**	**n=181**	**n=318**
I	98.2%	31.4%	60.7%	1.8%	73.3%	38.2%
J	98.5%	**3.6%**	**77.9%**	5.9%	80.9%	46.3%
K	98.5%	21.6%	61.5%	4.6%	85.0%	49.2%
L	95.7%	28.3%	68.8%	2.8%	42.9%	30.5%

* excluding deaths, **excluding death, lost-to follow-up and those declining participation, *** excluding those declining participation

Accumulation of HIV drug resistances with ART duration: evaluation in 12 decentralised centres in Cameroon. Article en cours de finalisation

Accumulation of HIV drug resistances with ART duration: evaluation in 12 decentralised centres in Cameroon

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Abstract

Background Although access to antiretroviral therapy (ART) has drastically increased in recent years in developing countries, access to viral load monitoring is still scarce especially in decentralized areas. Therapeutic failure is thus difficult to diagnose and patients are maintained on sub-optimal ART, increasing the risk to develop HIV drug resistances.

Methods Retrospective cohort study of all HIV-1 infected adults who initiated ART between June and December 2014, 2013 and 2012, in 12 decentralized primary care sites in Cameroon. Retention rate was estimated. Patients who were still in care 12, 24 and 36 months after ART initiation were invited to be virologicaly evaluated.

Results A total of 2,218 HIV-1 infected patients initiated ART during the three time periods considered in the 12 sites. Overall, the median age at ART initiation was 39 (IQR: 32-46) years and 69.9% patients were female. Retention was significantly lower at M36 (39.9%) than at M24 (53.5%) and M12 (63.3%) ($p<0.01$), as well as in men ($p=0.029$), younger patients ($p=0.014$), and patients with lower CD4 level at ART initiation ($p<0.001$). A total of 941 patients were virologicaly evaluated, the proportion of patients virologicaly suppressed was 66.6%, 62.7% and 59.8% at M12, M24 and M36, respectively and was not significantly different at the three time point evaluation. HIV DR was evaluated for 157 patients with a VL> 5000 cp/mL. The proportion of patients with >1 major mutation increased with time on ART ($p=0.03$).

Conclusion Retention, adherence to ART and virological suppression were alarmingly low in these 12 decentralized primary care sites. These results are very far from the last 90 in the UNAIDS targets of 90-90-90. This reflects the urgent need of improving Health care system in decentralized areas and especially VL to prevent maintaining patients in virological failure on inefficient ART and HIV DR acquisition.

Introduction

In 2016, 36.7 million [30.8-42.9] people worldwide were living with HIV and 20.9 million people were accessing antiretroviral therapy (ART) [1]. This number is constantly increasing due to the progressive relaxation of the immunological criterion to initiate ART, to a decrease of mortality but also due to an HIV incidence that remains high [2]. To fight the HIV epidemics, the 2016 WHO guidelines [3] waivered all criteria for ART initiation and recommended universal access to ART and routine viral load (VL) monitoring of patients on ART, in line with the UNAIDS 90-90-90 goals [4].

Since 2006, the recommended ART regimen have also evolved. In adults, the first line is still based on the combination of two nucleoside reverse-transcriptase inhibitor (NRTI) and one non-nucleoside

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reverse-transcriptase inhibitor (NNRTI). The two recommended NNRTI are still Efavirenz (EFV) and Nevirapine (NVP). For NRTI, Lamivudine (3TC) or Emtricitabine (FTC) must be combined with Tenofovir (TDF) or Zidovudine (AZT). Stavudine (d4T) was replaced by TDF in 2010 due to its toxicity.

The 2016 WHO guidelines recommend VL measurement at 6 months, 12 months after ART initiation and thereafter annually. Currently, virological failure is defined by a VL above 1000 copies/mL [3, 5-7]. However, viral load monitoring remains scarce in many settings, and care essentially relies on immuno-clinical monitoring, although it is now well established that clinical and immunological criteria do not adequately detect therapeutic failure [8, 9]. In Africa, the proportion of patients in virological failure can be alarmingly high [10, 11], and if patients who are failing on ART or who are poorly adherent are not identified, the risk to develop HIVDR is high [12-14]. The WHO has long been worried about the risk of HIVDR, and developed early warning indicators (EWIs) to monitor this risk at site levels.

In 2001, Cameroon set up a plan to widen ART access through decentralization to HIV care sites at a primary level of the health system pyramid at the district level [15]. These district HIV care sites are characterized by more limited technical and human resources [16] and very few of them are able to perform VL monitoring for patients on ART. Few studies performed virological evaluations in this context in Cameroon [10, 17]. In Cameroon, studies have estimated EWIs, and showed that the risk of emergence of HIVDR must be taken seriously [18, 19].

The current study aimed at evaluating the prevalence of virological failure, identifying the risk factors of virological failure on the one hand and of HIV DR acquisition on the other hand. To study the effect of duration on ART, three cohorts of patients were considered: patients on ART for 12, 24 or 36 months.

Materials and methods

Study design

The study was conducted in 12 HIV care sites selected from a list of all eligible HIV care sites established with the help of the National Committee of Fight against AIDS (CNLS) which respected the following: i) in 3 regions of Cameroon (Centre, East, and North-West) to cover a wide part of the country; ii) in decentralized areas and iii) with an active number of patients on ART between 100 and 500 to limit heterogeneity due to site capacity.

From September 2015 to January 2016, a cross-sectional evaluation was conducted to assess virological suppression at 12 (M12), 24 (M24) and 36 (M36) months of ART in patients on first-line ART. Patients were eligible if they were: HIV-1 positive, were 21 years of age or more at ART initiation, and were still on 1st line ART at the time of evaluation. At the time of evaluation, clinical data were recorded

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96 in a standardized case report form and adherence to ART was assessed by the number of missed doses
97 during the last 4 days [20], those reporting no missed-dose were categorized as 100% adherent.

98 Then, three retrospective cohorts were constituted from the medical charts of the 12 sites considering
99 all patients aged 21 or older at ART initiation and who initiated ART during the three time periods of
100 interest: June 2014 to February 2015, June 2013 to February 2014, and June 2012 to February 2013. A
101 standardized case report form was used to collect the following information: HIV-1 serology, date of
102 ART initiation, ART prescribed since initiation along with dates and reasons of modification, vital status
103 and date of vital status evaluation.

104 Retention rates at 12, 24 and 36 months were calculated as the proportion of patients who had neither
105 died nor were lost to follow-up (LTFU) before reaching these time-points among eligible patients.
106 Patients transferred to another care site before these time-points were excluded due to lack of
107 information regarding their continuity of care. In contrast, patients who did not participate in the study
108 but were still followed (patients who refused to participate, who sent a third party to take ART or for
109 whom the study was not proposed but who came for consultation) were included in the analysis as
110 still being monitored. Similarly, patients who were not eligible for virological evaluation, i.e. on second-
111 line ART at the time of the evaluation, were considered to be still followed.

112 Viral load quantification

113 During the 12, 24 and 36 month follow-up visit at the HIV care site, a 5 ml blood sample was drawn
114 and 5 whole blood spots of 50µl were deposited on each of two Whatman 903 filter paper cards. The
115 dried blood spot (DBS) cards were left to dry for 3 hours at ambient temperature before being
116 individually packed in sealed bags with two desiccants, and stored in a dedicated box at ambient
117 temperature. The DBS cards were transferred weekly to the IRD-CREMER virology laboratory in
118 Yaoundé (Cameroon) for VL measurement.

119 At arrival at the laboratory, DBSs were stored at -20°C until use for VL. For viral load measurement,
120 following the manufacturer's protocol, two spots of 50µl were cut and placed in 1.7mL of lysis buffer
121 provided in the Abbott m2000sp preparation kit, and was left for 30mn under constant agitation. Then,
122 1ml of the eluate was placed in a tube and centrifuged for 10mn at 2000rpm in order to remove small
123 paper particles from the supernatant, which is then used like plasma for RNA extraction and measure
124 of the VL using the m2000rt Abbott real-time PCR.

125 HIV DR genotyping protocol

126 HIV DR genotyping was measured in patients with a VL >5000 copies/mL. After RNA extraction as
127 described just above, the purified nucleic acids were then used for HIV-1 DR genotyping using an in-

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house protocol optimized for DBS testing. RT region was amplified using ANRS in-house protocol that amplifies a fragment of 798 bp (www.hivfrenchresistance.org/ANRS-procedures.pdf). PCR products were purified and directly sequenced using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Carlsbad, CA). HIV-1 subtyping was generated with MEGA 6 (https://www.megasoftware.net/). Thirteen Relevant drug resistance mutations (DRMs) in RT were identified using the Stanford interpretation algorithms v8.3 (https://hivdb.stanford.edu/). Sequences classified as susceptible and potential low-level resistant were considered to have no drug resistance.

Statistical analysis

Baseline characteristics were compared between ART initiation periods using chi-2 test for categorical variables and using ANOVA for continuous variables.

Retention rates were compared by duration on ART. Factors associated with retention were identified using logistic regression models.

Patients were considered virologicaly suppressed if their viral load was <1000 copies/mL. Factors associated with virological suppression were identified using logistic regression models. Factors investigated were demographic characteristics (age, gender), WHO clinical stage at ART initiation, adherence at evaluation and biological exams.

All statistical analysis were performed using Stata 14 (College Station, Texas, USA).

Ethical clearance

The protocol of the study was submitted in Cameroon to the National Ethics Committee of research for human health (CNERSH, ethical clearance number 2015/04/580/EC/CNERSH/SP), and in France to the Institutional Review Board (IRB) of Institut Pasteur in Paris (France). Written informed consent was obtained from each participant.

Results

Description of the eligible population

In the 12 sites, 2,218 HIV-1 infected patients initiated ART during the three time periods considered (Fig 1). Overall, the median (inter quartile range (IQR)) age at ART initiation was 39 (32-46) years and 1,550 (69.9%) patients were female, these characteristics being similar in the three cohorts (S1 Table). A majority of patients were from North-West region (45.2%), this proportion was significantly higher in the M36 cohort. The median (IQR) level of CD4 count at ART initiation significantly increased in the recent years (p<0.01). The proportion of patients for whom the initial ART was 3TC+TDF+EFV increased

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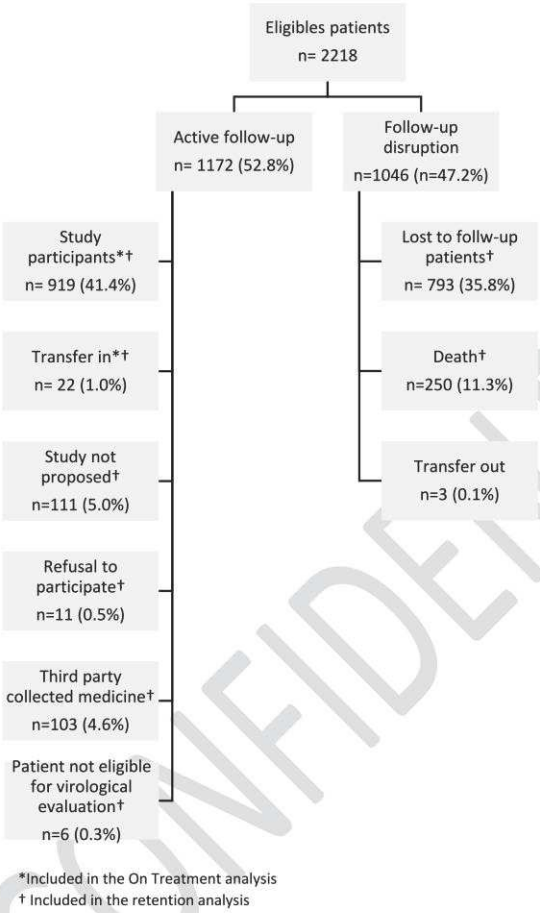
158 and represented about two-thirds on the M12 cohort. In the meantime, the proportion who initiated
159 3TC+AZT+NVP decreased.



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163 **Figure 1:** Flow diagram of study enrollment.

164 Retention

165 Of the 2,218 patients, three patients were transferred to another HIV care site and were not
166 considered in the calculation of retention rate. On the 2,215 patients considered, 1,172 (52.8%)
167 patients were still in care (Fig1). However, these proportions differed significantly between the three
168 cohorts (p<0.001) and were 63.3%, 53.5 and 39.9% in the M12, M24 and M36 cohorts, respectively (S2
169 Table).

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170 In multivariate analysis, the retention rate remained significantly lower as time on ART increased
171 (p<0.001) (Table 1). Men (p=0.029), younger patients (p=0.014), and patients with lower CD4 level at
172 ART initiation (p<0.001) were also less likely to remain in care.

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174 **Table 1.** Predictors of retention among eligible population of the study

Characteristic	N	Still in care (n=1172)	Adjusted OR (95% CI)	P
Month of evaluation				**<0.001**
M12	774	490 (63.3)	ref	
M24	791	423 (53.5)	**0.61 (0.48-0.75)**	
M36	650	259 (39.9)	**0.34 (0.27-0.43)**	
Gender				**0.029**
Female	1549	844 (54.5)	ref	
Male	665	328 (49.3)	**0.80 (0.65-0.98)**	
Age (Years)				**0.014**
21-30	436	208 (47.7)	ref	
31-40	843	439 (52.1)	1.2 (0.93-1.55)	
41-50	575	314 (54.6)	**1.4 (1.1-1.9)**	
>51	361	211 (58.5)	**1.55 (1.14-2.10)**	
CD4 count at ART initiation (/mm³)				**<0.001**
<50	257	114 (44.4)	ref	
50-99	213	109 (51.2)	1.35 (0.92-1.97)	
100-199	348	219 (62.9)	**2.16 (1.53-3.04)**	
200-349	447	297 (66.4)	**2.52 (1.81-3.49)**	
350-499	134	83 (61.9)	**1.78 (1.15-2.77)**	
≥500	67	47 (70.2)	**2.80 (1.50-5.22)**	
NNRTI molecule at initiation				0.40
EFV	1301	710 (55.0)		
NVP	894	443 (50.1)		
NRTI molecule at ART initiation				
ZDV	944	478 (51.4)		
TDF	1238	664 (53.9)		
D4T	24	15 (62.5)		

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176 Characteristics of patient virologicaly evaluated

177 A total of 941 patients were virologicaly evaluated (Fig 1). The median (IQR) of age of participant was
178 39 (33-46). The majority were women (72.9%), with a low level of education (primary or no education)
179 (59.1%), and were farmers (36.5%) (Table 2 and S3 Table). Almost half of the participants recognized
180 resorting to traditional medicine (41.7%) and purchasing drugs without medical prescription (45.0%).

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182 **Table 2.** Characteristics of patients with virological evaluation

Characteristic	M12 (n=398)	M24 (n=339)	M36 (n=204)	P
Region of follow-up				**<0.01**
Centre	113 (28.4)	134 (39.5)	53 (26.0)	
North-West	185 (46.5)	140 (41.3)	107 (52.5)	
East	100 (25.1)	65 (19.2)	44 (21.6)	
Gender				0.23
Female	296 (74.4)	236 (69.6)	154 (75.5)	
Male	102 (25.6)	103 (30.4)	50 (24.5)	
Age (Years)				
Median (IQR)	39 (32-46)	40 (34-48)	40 (33.5-46)	0.32
NNRTI molecule used at ART initiation				**<0.01**
EFV	291 (73.1)	198 (58.4)	98 (48.0)	**<0.01**
NVP	106 (26.3)	140 (41.3)	103 (50.5)	
Missing data	1 (0.3)	1 (0.3)	3 (1.5)	
NRTI molecule used at ART initiation				**<0.01**
TDF	283 (71.1)	176 (51.9)	87 (42.7)	
ZDV	112 (28.1)	161 (47.5)	114 (55.9)	
D4T	2 (0.5)	1 (0.3)	2 (1.0)	
Missing data	1 (0.25)	1 (0.29)	1 (0.5)	
Level of CD4 count at ART initiation (cells/mm³)				0.26
Median (IQR)	202 (94-316)	195 (104-305)	201 (125-300)	
Missing data	99 (24.9)	70 (20.6)	47 (23.0)	
Characteristics at virological evaluation				
BMI (kg/m²)				0.09
Median (IQR)	22.8 (20.5-25.0)	23.7 (20.8-26.1)	23.3 (20.7-25.7)	
Missing data	4 (1.01)	1 (0.3)	2 (1.0)	
WHO stage				0.46
Stage 1-2	312 (78.4)	277 (81.7)	160 (78.4)	
Stage 3-4	73 (18.3)	55 (16.3)	42 (20.6)	
Missing data	13 (3.3)	7 (2.1)	2 (1.0)	
NNRTI molecule used at the moment of evaluation				**<0.01**
EFV	302 (75.9)	218 (64.3)	126 (61.8)	
NVP	96 (24.1)	120 (35.4)	78 (38.2)	
Missing data	0 (0)	1 (0.3)	0 (0)	
NRTI molecule used at the moment of evaluation				**0.02**
TDF	294 (73.9)	219 (64.6)	127 (62.3)	
ZDV	102 (25.6)	119 (35.1)	76 (37.3)	
d4T	2 (0.5)	0 (0)	1 (0.5)	
Missing data	0 (0)	1 (0.3)	0 (0)	
Drug substitution				**<0.01**
No	368 (92.5)	252 (74.6)	129 (63.2)	
Yes	29 (7.3)	87 (25.4)	75 (36.8)	
Missing data	2 (0.3)	-	-	
Latest CD4 count level (cells/mm³)				**<0.01**
Median (IQR)	300 (188-495)	380 (262-590)	401 (244-566)	
Missing data	130 (32.7)	72 (21.3)	47 (23.0)	

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At the time of evaluation, the most common ART regimens were 3TC+TDF+EFV and 3TC+AZT+NVP. The proportion of ART regimen including Efavirenz (EFV) was significantly higher at M12 than at M24 and M36, whether at the time of treatment initiation or at the time of evaluation. Regarding NRTI use, Tenofovir was the more commonly used at the moment of ART initiation (58.2%) as at the moment of the evaluation (68.1%); its use was significantly more frequent for patients at M12 than at M24 and M36. The proportion of patients with at least one change within first-line ART significantly increased from 7.3% at M12 to 36.8% at M36 ($p=0.01$). The most frequent reason for treatment modification was an ART stock out (79.4%), other reasons were adverse reactions (9.2%), tuberculosis (2.7%), pregnancy (2.2%), non-adherence (1.1%), suspicion of treatment failure (1.0%) and co-infection with HIV-2 (0.5%).

At ART initiation, CD4 level didn't differ by cohorts. On the other hand, CD4 level was significantly higher in the M24 and M36 cohorts than in the M12 cohort ($p<0.01$).

Virological suppression

The proportion of patients virologicaly suppressed was stable ($p=0.15$) and was 66.6%, 62.7% and 59.8% at M12, M24 and M36, respectively (Table 3).

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212 **Table 3:** Virological outcomes according to the time spent on ART

Characteristics	M12	M24	M36	p
Viral load level (copies/mL) (n=937)				0.15
<1000	265 (66.6)	213 (62.8)	121 (59.3)	
>=1000	130 (32.7)	125 (36.9)	83 (40.7)	
HIV DR genotyping (VL >5000 copies/mL)	82 (20.6)	82 (24.2)	54 (26.5)	
Amplification success	60 (73.2)	59 (72.0)	38 (70.4)	0.94
One major mutation or more affecting NNRTI (n=60)	44 (73.3)	44 (74.6)	30 (79.0)	0.82
One major mutation or more affecting NRTI (n=60)	32 (53.3)	35 (59.3)	29 (76.3)	0.07
Number of major mutation				**0.03**
Median (IQR)	2 (1-4)	2 (0-4)	4 (2-4)	
Number of major NNRTI mutation				0.15
Median (IQR)	1 (0-2)	1 (0-2)	2 (1-2)	
Number of major NRTI mutation				**<0.01**
Median (IQR)	1 (0-2)	1 (0-2)	2 (1-2)	
NNRTI resistance	43 (71.7)	43 (72.9)	30 (79.0)	0.71
NVP	43 (71.7)	43 (72.9)	30 (79.0)	0.71
EFV	43 (71.7)	43 (72.9)	30 (79.0)	0.71
RPV	22 (36.7)	31 (52.5)	25 (65.8)	**0.02**
ETR	14 (23.3)	21 (35.6)	20 (52.3)	**0.01**
NRTI resistance				
any	32 (53.3)	35 (59.3)	29 (76.3)	0.07
xTC	30 (50)	35 (59.3)	29 (76.3)	**0.04**
TDF	18 (30)	18 (30.5)	19 (50)	0.09
ZDV	7 (11.7)	8 (13.6)	15 (39.5)	**<0.01**
DDI	24 (40)	23 (39.0)	23 (60.5)	0.08
Patient with TDF and ZDV resistance	4 (6.7)	7 (11.9)	12 (31.6)	**<0.01**

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215 In multivariate analysis, virological suppression was significantly more likely in married patients and in
216 patients who initiated ART with >350 CD4/mm^3 (adjusted OR [95% CI]: 1.56 [1.04-2.34] and 2.45 [1.36-
217 4.39], respectively) (Table 4). On the other hand, virological suppression was significantly lower in
218 patients living >30 Km from the HIV care site and in patients in immunological failure (adjusted OR
219 [95% CI]: 0.63 [0.41-0.99] and 0.40 [0.27-0.60], respectively).

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224 **Table 4:** Risk factors of virological suppression (n=937)

Characteristics	N	Virological Suppression n (%)	p	Adjusted OR (95% CI)
Month of evaluation			0.15	
M12	395	265 (67.1)		
M24	338	213 (63.0)		
M36	204	121 (59.3)		
Region of follow-up			**0.003**	
Centre	300	210 (70.0)		
North-West	429	274 (63.9)		
East	208	115 (55.3)		
Gender			0.33	
Female	683	443 (64.9)		
Male	254	156 (61.5)		
Age (Years)			0.16	
21-30	167	100 (59.9)		
31-40	346	212 (61.3)		
41-50	258	177 (68.6)		
>51	166	110 (66.3)		
Marital status			**0.014**	
Never married	288	170 (59.0)		ref
Married	435	301 (69.2)		**1.56 (1.04-2.34)**
Divorced	80	44 (55.0)		0.72 (0.40-1.30)
Widowed	123	79 (64.3)		0.93 (0.54-1.60)
Unspecified	11	5 (45.5)		Non estimable
Educational level			0.29	
Primary school or none	547	342 (62.5)		
Upper than primary school	390	257 (65.9)		
Distance between household and HIV care site			0.12	
Less than 10 Km	354	238 (67.2)		ref
Between 10 and 30 Km	234	152 (65.0)		0.71 (0.46-1.10)
More than 30 Km	252	156 (65.9)		**0.63 (0.41-0.99)**
Unspecified	97	53 (54.6)		0.45 (0.26-0.80)
Use of traditional medicine			0.029	
No	488	330 (67.6)		
Yes	348	213 (61.2)		
Unspecified	101	56 (55.5)		
Purchase of drugs outside hospital pharmacy			0.06	
No	460	308 (67.0)		
Yes	378	237 (62.7)		
Unspecified	99	54 (54.5)		
NNRTI molecule at initiation			**0.041**	
EFV	584	388 (66.4)		
NVP	348	208 (59.7)		
NRTI molecule at initiation			0.31	
ZDV	386	239 (61.9)		
TDF	543	355 (65.4)		
D4T	5	3 (60.0)		

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231 **Table 4 (continuation):** Risk factors of virological suppression (n=937)

Characteristics	N	Virological Suppression n (%)	p	Adjusted OR (IC 95%)
CD4 count at ART initiation (/mm³)			**0.002**	
<200	362	231 (63.8)		ref
200-350	249	166 (66.7)		1.12 (0.76-1.65)
≥350	111	55 (76.4)		**2.45 (1.36-4.39)**
Not available	215	117 (54.5)		1.82 (0.75-4.39)
BMI at evaluation (Kg/m²)			**0.039**	
<18.5	72	38 (52.8)		
18.5-25	572	358 (62.6)		
25-30	221	155 (70.1)		
≥30	65	45 (69.3)		
Not available	7	3 (42.9)		
WHO stage			**0.003**	
1-2	745	497 (66.7)		Ref
3-4	170	93 (54.7)		0.65 (0.42-1.01)
Immunological failure*			**<0.01**	
No	534	382 (71.5)		ref
Yes	154	86 (55.8)		**0.40 (0.27-0.60)**
NNRTI molecule in the current ART regimen			0.29	
EFV	643	418 (65.0)		
NVP	293	180 (61.4)		
NRTI molecule in the current ART regimen			0.30	
ZDV	296	181 (61.1)		
TDF	637	416 (65.3)		
D4T	3	1 (33.3)		
Drug substitution			0.99	
No	745	476 (63.9)		
Yes	191	122 (63.9)		
Self-reported number of missed doses during the last 4 days			**<0.01**	
None	592	399 (68.1)		
More than one time	331	193 (57.3)		
Knowledge of HIV status by someone other than the patient				
Spouse			0.08	
Yes	353	238 (67.4)		
No	584	361 (61.8)		
Family (except Spouse)			0.56	
Yes	624	403 (64.6)		
No	313	196 (62.6)		
Friends			0.80	
Yes	83	52 (62.7)		
No	854	547 (64.1)		
Nobody			0.90	
Yes	51	33 (64.7)		
No	886	566 (63.9)		

232 *Defined by CD4 count below the level measured at ART initiation or below 100/mm³ (WHO 2015)[3]

HIV-1 drug resistance in patients in virological failure

HIVDR was investigated in 218 (23.2%) patients who had a VL above 5000 copies/mL, and was successful in 157 of them (72.0%) (Table 3), this proportion didn't differ according to the duration of ART exposure (p=0.94).

Regarding HIV subtype, 104 (66.2%) was CRF02_AG subtype (S4 Table).

The most common mutation was K103N (54.8%). Only one mutation (G190A) was significantly more frequent when time on ART increased (S4 Table). The proportion of patients with a virus harboring at least one major mutation affecting NNRTI was not associated with the duration on ART (p=0.82). However, the proportion of patients with a virus resistant to RPV or ETR was significantly higher at M36 than at M24 and M12 (p=0.02 and p=0.01, respectively).

The proportion of patient with a virus harboring at least one major mutation affecting NRTI was 53.3% at M12 and increased to 59.3% and 76.3% at M24 and M36, respectively (p=0.03). The proportion of patients with a virus resistant to xTC and ZDV were significantly higher at M12 than at M24 and M36 (Table 3). The most frequent mutation was M184V (56.7%). Mutations T215C/F/I/S/Y were observed in 27 patients (17.2%) and the K65 mutation was observed in 25 patients (25%). The proportion of patients with M184V, T215C/F/I/S/Y, D67N and K70R were significantly higher for patient at M36 than at M24 and M12. A total of 23 patients had a virus resistant to TDF and AZT. This proportion was significantly higher at M36 (31.6%) than at M24 (11.9%) and M12 (6.7%) (p<0.01).

In multivariate analysis, education level, latest CD4 count level, NRTI molecule used in the current ART regimen, adherence and non-disclosure if HIV status increased the risk of having one or more mutation affecting NRTI. The proportion of patient having one or more major mutations affecting NRTI was significantly higher in patient with an educational level upper than primary school (OR 2.33 IC95% [1.04-5.20]). This proportion was significantly lower in patients with CD4 count upper than 350/mm^3 (OR 0.21 IC95% [0.06-0.70]) and in patient with TDF in their current ART regimen compared to ZDV (OR: 0.32 [0.14-0.75]).

Discussion

This study was conducted in 12 decentralized primary care sites from 3 regions of Cameroun with the highest HIV prevalence [15]. The study setting was very rural, which is illustrated by a low access to education beyond primary school and a high proportion of farmers. Comparison of the three cohorts (M12, M24 and M36) also illustrates the raising of the CD4 threshold for ART initiation, and the changes of guidelines regarding recommended first-line ART [7, 21]. The number of patients transferred to another HIV care sites was low which could mean that few patients were transferred due to a switch

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265 to a second line of ART. Knowing that delayed switch to second-line ART after failure increased the risk
266 of future failure, it's is consistent with a high rate of virological failure observed in our study [22]. The
267 lack of virological monitoring should play an important role in this situation.

268 Overall, retention was low and was logically significantly decreasing with the duration of ART exposure.
269 The retention rate at M12 (63.3%) was consistent with previous reports in Cameroon [18, 19, 23-26].
270 Only few studies reported retention rates beyond 12 months of ART. Retention rates at 24 and 36
271 months were 53.5% and 39.9%, respectively. This is in line with previous studies in Cameroon [27].
272 [25]. As already reported, retention was worse in men. [24, 25, 28-31]. It is interesting to note that
273 Koole and col noted in their study that the difference between genders was only observed in sites
274 without community distribution of ARV drugs [31] and it was the case in our study. As previously
275 reported also we reported a lower retention rate for younger adults and lower CD4 count at ART
276 initiation.

277 In univariate analysis, EFV was found to be associated with a better retention. However, this was
278 explained by the higher proportion of patients who initiated EFV-based ART in recent years, as the
279 association disappeared after adjusting for the duration on ART.

280 The virological evaluation was proposed to all patients still actively followed, and who fulfilled the
281 criteria. The proportion of patients who did not participate was low, and did not differ between the
282 three ART duration cohorts.

283 The rate of virological suppression at the threshold of 1000 copies/ml was low, even at 12 months of
284 ART, and was around 60% whatever the duration on ART. This was lower than usually reported in Sub-
285 Saharan Africa [10, 32-34], and also lower than in previous studies in Cameroon which reported higher
286 virological suppression rate at the same threshold at M12 (from 90.7% to 76.4%) and M24 (from 86.1%
287 to 77.5%) [17, 26, 35-37] and at M36 (82.4%) [38]. However, these studies have essentially been
288 performed in HIV care site in large urban centers in which HIV studies are regularly conducted. Thus,
289 both care staff and patients may be more exposed to trainings and to scientific results, which could
290 improve management, adherence and hence virological suppression. However, some countries of
291 West Africa reported similar results for patients on first-line of ART as in Abidjan, Ivory Coast with 25%
292 of patient with of detectable VL at 12 months [12] or a high rate of VF of 30.8% Lomé, Togo, for one
293 year (10–14 months) [39]. One study realized in a district hospital located in a remote area in north
294 Cameroon also reported similar results with a rate of 61.2% virological suppression at M12 and 59.6%
295 at M24 [40].

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296 The low proportion of patients virologicaly suppressed could also be partly explained by the fact that
297 this evaluation was conducted on a single viral load measurement, without a confirmatory viral load
298 after strengthening of adherence in case of initial failure.

299 Risks factors of virological failure reported were being on clinical and immunological failure according
300 to WHO definition. These results are fully consistent. We reported a rate of 45.3% and 44.2% of
301 patients with virological failure with clinical and immunological failure respectively. These results fit
302 well with result previously reported of poor sensibility performance for antiretroviral treatment failure
303 detection of clinical and immunological criteria [9, 41-43].

304 Regarding HIV DR, All patients with antiretroviral drug resistance were resistant to NNRTIs, and three
305 quarters of them were also resistant to NRTIs as previously described [17]. The proportion of patient
306 with NNRTI mutation was slightly higher than previously reported and the proportion of patient with
307 NRTI mutations was similar [37, 44, 45].

308 As previously described K103N and M184V were the most frequent mutations found in RT (54.8% and
309 56.7%, respectively) [37] due to the systematic use of the 3TC, the predominant use of EFV and the
310 low genetic barrier of these mutations [46-48]. The relatively high rate of K65R (15.9%) is the corollary
311 of the increased use of TDF following WHO guidelines. The prevalence of TDF resistance is now known
312 to be high in sub-Saharan Africa [49]. It is interesting to note that in our study no patient had a HIV
313 subtype C. A high rate of resistance of second-generation NNRTIs (rilpivirine and etravirine) was
314 observed up to more than 50% of patients with VL >5000 copies/mL at 24 months of ART. This
315 observation can be explained on the one hand by the presence of Y181C mutation linked to the use of
316 NVP and the of the H221Y mutation [46, 50, 51]. On the other hand, the resistance associated mutation
317 accumulation explained around 60% of the ETR resistance. This situation, which has been previously
318 described, jeopardizes the use of these molecules in third or fourth line of ART [17, 39, 44, 52-55].

319 Interestingly, 25% of the patients with a VL >5000 copies/mL exhibited no HIV DR This proportion was
320 similar whatever the duration on ART. One can assume that these patients have a poor adherence, and
321 could have been virologicaly suppressed after strengthening of adherence. Still this would only
322 marginally increase the proportion of patients virologicaly suppressed.

323 Worryingly, 23 (2.4% off all evaluated patients) acquired resistance to the three available NRTI
324 recommended in second-line ART, and the proportion of patients concerned significantly increased
325 with the duration on ART. If these patients were switched to the standard second-line ART would in
326 fact be on a monotherapy of boosted protease inhibitor.

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327 Thus, our study confirms that maintaining ART effectiveness is challenging especially in remote areas
328 in Cameroon. This might be due to patient-related factors such as longer distance and time to reach
329 hospital, lower incomes, lower literacy, and poorer living conditions in rural settings. This might come
330 also from healthcare-related factors such as poorer knowledge and experience of healthcare workers
331 in HIV/AIDS management and ART use, greater staff shortage and turn-over, poorer laboratory
332 infrastructures (to monitor ART effectiveness and adverse events), and higher frequency of drug stock-
333 outs. In Cameroon, Fokam and col described poorer performances in terms of retention in rural than
334 in urban setting [19]. Moreover, a recently published study evaluating viral suppression at M12-24 in
335 19 urban and 10 rural clinics (different than those included in our study), reported similar results to
336 ours with a virological suppression rate of 72.1% (95% CI: 66.3–77.2), a lower virological suppression
337 rate in rural than in urban areas and a high proportion of K65R mutation [56]. However, some studies
338 reported better results in decentralized areas than compared to centralized [16, 57] and
339 decentralization of care not appeared to be a risk factor of virological failure [10]. This heterogeneity
340 of results could be linked to the definition of "decentralized" or "rural". For example, Zoulafy et al
341 reported result coming from rural area in Cameroon but patients were followed in a Regional hospital
342 contrarily to our study in which all patients were followed at district level and in rural setting [37]. In
343 the same way, term "decentralized" is used to define peripheral health centres or lower whatever if
344 these centres are in rural or urban setting and all may vary in meaning between countries [58, 59].

345 Our findings should be interpreted taking into account several study limitations. Regarding the
346 retention rate measurement, in rural setting a good registry keeping is slightly challenging. Aware of
347 that, we realized three successive audit of registers and excluded any duplicate or doubtful record.
348 There was a number of patients who sent a third person (family member) to the HIV care site for taking
349 their ART during the study period (corresponding to a duration of three successive consultations). That
350 practice is known of the HIV care providers and we decided to integrate these patients to the count of
351 patient linked to care. However, we didn't collect any information about the reasons of not coming in
352 person to the consultation. Use of DBS for VL measurement could generate some skepticism especially
353 in term of false positive patient due to an amplification of proviral DNA. However, technic use for VL
354 measurement have a sensibility of 96% at the threshold of 1000 copies/mL. Specificity of the technic
355 being evaluated to 96% also, thus the impact of using DBS for VL measurement in term of proportion
356 of patient under or below the threshold of 1000 copies/mL should be negligible.

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Conclusion

Our study is the biggest study reported data coming from remote areas in Cameroon. We described here an alarming situation ten years after ART introduction in the country and which is very far from the 90-90-90 objectives. Increase of life expectancy, transmitted HIV DR rate and number of patient on ART can only reinforce this worry. This description highlighted the need of strengthening the health care system. Improvement of retention rate required especially community care involvement and could be help by computerization of the health care system. Virological suppression rate and prevention of HIV DR acquisition required urgently access to VL monitoring and an adapted adherence support for patients on ART.

Acknowledgments

We sincerely thank all the patients who agreed to take part in this study, the clinical and laboratory staff from the three HIV care sites who participated in this study: Dr Dongmeza Donald, Ngono Olivier, Dr Kamdom Nicolas, Mrs. Nkodo M. Roanne, Dr Misse Misse P., Dr Ihindi, Dr Ngo Nsoga Marie, Mrs. Ymele Doris, Dr Wandji Kounougo, Mr.Nde Rene Chi, Dr Eugene Chiabi, Mrs. Young Augustine, Dr Bawe Edwin, Mr. Njoya Mamouda, Dr Souore Sanda Jessica, Dr. Essono Mebouinz, Mr. Tize Gabriel, Dr Nyankoue Mebouinz Ferdinand, Zima Mikok Parfait Cyrille, Dr Abeng Brigitte, Mr. Tsague Ngueffo Achille Bosco . We sincerely thanks the surveyors of Care and Health Program organization: Mr. Belinga Henri Louis, Mr. Sinsaï Ronald Nguelewou, Mr. Zamo Alain John, Mr.Matike Ndoumbe René William and Mr. Kamga Eric for his technical support and Mrs. Zobo Atangana Marie Charlotte for her help regarding data entry. We thanks Dr Tsomo Zephirin of Yaounde Central Hospital and Colonel Mpoudi Ngole E. of CREMER. We also are very grateful to the Central Technical Group of the National AIDS Control Committee and the Division of Operational Health Research of the Ministry of Public Health for their support and their help in the implementation of the study. We also thank the Clinical Core from the Center for Translational Research at Institut Pasteur (Paris France) and more specifically Mrs. Cassandre Von Platen for her valuable help in the ethical and regulatory procedures. We thanks Cameroonian office of Expertise France and especially Dr Akamba Jean Jacques. We thanks Expertise France (previously named ESTHER) for their financial support.

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628 Supporting information

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630 **S1 Table.** Sociodemographic and clinical characteristics of the patients included in the study

631 **S2 Table**. Status of the patients included in the study at the moment of evaluation

632 **S3 Table**. Characteristics of patients with virological evaluation

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S1 Table. Sociodemographic and clinical characteristics of the patients included in the study

Characteristics	M12 (n=774)	M24 (n=791)	M36 (n=653)	p
Region				**<0.01**
Centre	189 (24.2)	226 (28.6)	134 (20.5)	
North-West	330 (42.6)	340 (43.0)	333 (51.0)	
East	255 (33.0)	225 (28.5)	186 (28.5)	
Age (years)				0.08
Median (IQR)	39 (32-46)	39 (33-47)	38 (32-44)	
Sex				0.43
Female	552 (71.3)	538 (68.0)	460 (70.4)	
Male	222 (28.7)	252 (31.9)	193(29.6)	
Missing data	0(0)	1(0.1)	0(0)	
CD4 count at ART initiation (/mm³)				**<0.01**
Median (IQR)	187 (79-318)	178 (78-284)	163 (62-274)	
Not available	270 (34.9)	254(32.1)	224 (34.3)	
Initial ART regimen				**<0.01**
3TC+TDF+EFV	514 (66.4)	348 (44.0)	205 (31.4)	
3TC+AZT+NVP	134 (17.3)	294 (37.2)	281 (43.0)	
3TC+AZT+EFV	53 (6.9)	82 (10.4)	83 (12.7)	
3TC+TDF+NVP	59 (7.6)	43 (5.4)	59 (9.0)	
3TC+d4T+NVP	2 (0.3)	11 (1.4)	3 (0.5)	
3TC+d4T+EFV	1 (0.1)	2 (0.3)	5 (0.8)	
Not specified	11 (1.4)	11 (1.4)	17 (2.6)	
Latest ART regimen registered				**<0.01**
3TC+TDF+EFV	533 (68.9)	410 (51.8)	284 (43.5)	
3TC+AZT+NVP	125 (16.2)	243 (30.7)	242 (37.1)	
3TC+AZT+EFV	47 (6.1)	52 (6.6)	48 (7.4)	
3TC+TDF+NVP	52 (6.7)	48 (6.1)	58 (8.9)	
3TC+d4T+NVP	2 (0.3)	10 (1.3)	2 (0.3)	
3TC+d4T+EFV	2 (0.3)	2 (0.3)	5 (0.8)	
Not specified	13 (1.7)	26 (3.3)	14 (2.1)	

Data are n (%) when nothing is specified. IQR : InterQuartile Range

S2 Table. Status of the patients included in the study at the moment of evaluation

Characteristics	M12 (n=774)	M24 (n=791)	M36 (n=653)
Active follow-up*	490 (63.3)	423 (53.5)	259 (39.9)
Study participants	392 (50.7)	329 (41.6)	198 (30.3)
Transfer in	6 (0.8)	10 (1.3)	6 (0.9)
Study not proposed	37 (4.8)	52 (6.6)	22 (3.4)
Refusal to participate	3 (0.4)	2 (0.3)	6 (0.9)
Third person sent for medical consultation	52 (6.7)	25 (3.2)	26 (4.0)
Patient not eligible for virological evaluation	0 (0)	5 (1)	1 (0.2)
Follow-up disruption	284 (36.7)	368 (46.5)	391 (60.2)
Lost to follow-up	219 (28.3)	272 (34.4)	302 (46.3)
Death	65 (8.4)	96 (12.1)	89 (13.6)
Transfer out	0 (0)	0 (0)	3 (0.5)

Data are n (%) when nothing is specified.

*Patients transferred to another HIV care site were not considered in the proportion calculation

S3 Table. Characteristics of patients with virological evaluation

Characteristic	M12 (n=398)	M24 (n=339)	M36 (n=204)	P
Marital status				0.12
Never married	119 (29.9)	117 (34.5)	54 (26.5)	
Married	187 (47.0)	160 (47.2)	90 (44.1)	
Divorced	33 (8.3)	21 (6.2)	26 (12.8)	
Widowed	54 (13.6)	39 (11.5)	30 (14.7)	
Missing data	5 (1.3)	2 (0.6)	4 (2.0)	
Educational level				0.59
Primary school or none	230 (57.8)	191 (56.3)	128 (62.8)	
Upper than primary school	164 (41.2)	146 (43.1)	75 (36.8)	
Missing data	4 (1.0)	2 (0.6)	1 (0.5)	
Occupation				0.24
Farmer	137 (34.4)	129 (38.1)	77 (37.8)	
Public servant	25 (6.3)	32 (9.4)	25 (12.3)	
Merchant	115 (28.9)	83 (24.5)	53 (26.0)	
Housewife	103 (25.9)	82 (24.2)	43 (21.1)	
Unemployed	7 (1.8)	3 (0.9)	3 (1.5)	
Student	7 (1.8)	4 (1.2)	2 (1.0)	
Retired	1 (0.3)	0 (0)	1 (0.5)	
Other	1 (0.3)	5 (1.5)	0 (0)	
Missing data	2 (0.5)	1 (0.3)	0 (0)	
Distance between household and HIV care site				**0.04**
Less than 10 km	170 (42.7)	122 (36.1)	64 (31.4)	
Between 10 and 30 km	88 (22.1)	88 (26.0)	58 (28.4)	
More than 30 km	110 (27.6)	89 (26.0)	55 (27.0)	
Missing data	30 (7.5)	40 (11.8)	27 (13.2)	
Use of traditional medicine				**0.02**
No	202 (50.8)	189 (55.6)	99 (48.5)	
Yes	165 (41.5)	109 (32.3)	76 (37.3)	
Missing data	31 (7.8)	41 (12.1)	29 (14.2)	
Purchase of drugs outside hospital pharmacy				0.20
No	203 (51.0)	164 (48.5)	96 (47.1)	
Yes	164 (41.2)	135 (39.6)	80 (39.2)	
Missing data	31 (7.8)	40 (11.8)	28 (13.7)	
ART regimen at initiation				**<0.01**
3TC+TDF+EFV	261 (65.6)	155 (45.7)	75 (36.8)	
3TC+AZT+NVP	83 (20.9)	118 (34.8)	91 (44.6)	
3TC+AZT+EFV	29 (7.3)	43 (12.7)	22 (10.8)	
3TC+TDF+NVP	22 (5.5)	21 (6.1)	11 (5.4)	
3TC+d4T+NVP	1 (0.2)	1 (0.3)	1 (0.5)	
3TC+d4T+EFV	1 (0.2)	-	1 (0.5)	
Missing data	1 (0.3)	1 (0.3)	3 (1.5)	

S3 Table (continuation). Characteristics of patients with virological evaluation

Characteristic	M12 (n=398)	M24 (n=339)	M36 (n=204)	P
Characteristics at the moment of evaluation				
ART regimen				**0.02**
3TC+TDF+EFV	275 (69.1)	195 (57.5)	115 (56.4)	
3TC+AZT+NVP	76 (19.1)	96 (28.3)	66 (32.3)	
3TC+AZT+EFV	26 (6.5)	23 (6.8)	10 (4.9)	
3TC+TDF+NVP	19 (4.8)	24 (7.1)	12 (5.9)	
3TC+d4T+NVP	1 (0.2)	0 (0)	0 (0)	
3TC+d4T+EFV	1 (0.2)	0 (0)	1 (0.5)	
Missing data	0 (0)	1 (0.3)	0 (0)	
ART modification				**<0.01**
No	368 (92.5)	252 (74.6)	129 (63.2)	
Yes	29 (7.3)	87 (25.4)	75 (36.8)	
Missing data	2 (0.3)	0(0)	0 (0)	
Number of drug substitution since ART initiation				**<0.01**
Median (IQR)	2 (1-2)	2 (1-2)	2 (1-2)	
Reason of ART modification				
Shortage of ART stock	15 (51.7)	63 (72.4)	61 (81.3)	**0.01**
Adverse reactions	7 (24.1)	9 (10.3)	1 (1.3)	**<0.01**
Option B +	2 (6.9)	1 (1.2)	1 (1.3)	**0.13**
Tuberculosis	2 (6.9)	0 (0)	3 (4.0)	0.08
Treatment failure suspicion	0(0)	1 (1.2)	1 (1.3)	0.17
Non adherence	1 (3.5)	1 (1.2)	0 (0)	0.28
HIV-2 co-infection	0 (0)	0 (0)	1 (1.3)	0.47
Missing data	2 (6.9)	12 (13.8)	7 (9.3)	0.98
Last CD4 count level (cells/mm³)				**<0.01**
Median (IQR)	300 (188-495)	380 (262-590)	401 (244-566)	
<50	10 (2.5)	8 (2.4)	9 (4.4)	
50-99	17 (4.3)	6 (1.8)	4 (2.0)	
100-199	48 (12.1)	26 (7.4)	17 (8.3)	
200-349	76 (19.1)	73 (21.6)	36 (17.7)	
350-499	52 (13.1)	58 (17.2)	33 (16.2)	
>=500	65 (16.3)	96 (28.4)	58 (28.4)	
Missing data	130 (32.7)	72 (21.3)	47 (23.0)	
Self-reported number of missed doses during the last 4 days				0.80
More than one time	151 (37.9)	118 (34.8)	71 (34.8)	
None	240 (60.3)	216 (63.7)	131 (64.2)	
Missing data	7 (1.8)	5 (1.5)	2 (1.0)	
Knowledge of HIV status by someone other than the patient				
Spouse	153 (38.4)	130 (38.5)	72 (35.3)	0.72
Family (except Spouse)	270 (67.8)	217 (64.0)	139 (68.1)	0.45
Friends	25 (6.3)	28 (8.3)	31 (15.2)	**< 0.01**
Nobody	25 (6.3)	19 (5.6)	7 (3.4)	0.34
Missing data	30 (7.5)	38 (11.2)	25 (12.3)	

S4 Table : HIV subtype and resistance mutations detected by drug class in patients failing ART following 12, 24 and 36 months of treatment with VL>5000cp/mL and success of HIV DR genotyping

	M12 (n=60)	M24 (n=59)	M36 (n=38)	p
NNRTI mutation				
any	44 (73.3)	44 (74.6)	30 (79.0)	0.82
G190A/E/S/V	5 (8.3)	6 (10.2)	11 (29.0)	**<0.01**
L100I	2 (3.3)	3 (5.1)	4 (10.5)	0.32
K101E	1 (1.7)	5 (8.5)	5 (13.2)	0.08
K103N/S	34 (56.7)	33 (56.0)	19 (50.0)	0.79
V106M/A	2 (3.3)	2 (3.4)	1 (2.6)	0.97
E138A/G/Q	3 (5.0)	5 (8.5)	5 (13.2)	0.36
Y181C/V	8 (13.3)	8 (13.6)	6 (15.8)	0.94
Y188C/L/H	4 (6.7)	4 (6.8)	1 (2.6)	0.64
P225H	6 (10.0)	9 (15.3)	7 (18.4)	0.48
M230L	3 (5.0)	4 (6.8)	1 (2.6)	0.66
H221Y	4 (6.7)	6 (10.2)	1 (2.6)	0.36
V179L/T	-	-	2 (5.3)	0.04
F227C	1 (1.7)	-	-	0.44
A98A/G	4 (6.7)	8 (13.6)	8 (21.1)	0.11
K101H/P	-	1 (1.7)	3 (7.9)	**0.05**
NRTI mutation				
any	34 (56.7)	35 (59.3)	29 (76.3)	0.12
M184V/I	26 (43.3)	34 (57.6)	29 (76.3)	**<0.01**
K65R	11 (18.3)	10 (17.0)	4 (10.5)	0.57
K70E	3 (5.0)	4 (6.8)	3 (7.9)	0.84
T215C/F/I/S/Y	6 (10.0)	7 (11.9)	14 (36.8)	**<0.01**
L74V/I	3 (5.0)	5 (8.5)	6 (15.8)	0.19
Y115F	1 (1.7)	3 (5.1)	-	0.26
D67N	3 (5.0)	1 (1.7)	8 (21.1)	**<0.01**
K70R	3 (5.0)	2 (3.4)	6 (15.8)	**0.05**
K219E/Q	6 (10.0)	4 (6.8)	6 (15.8)	0.36
T69D	-	1 (1.7)	1 (2.6)	0.49
L210W	1 (1.7)	1 (1.7)	3 (7.9)	0.17
E44D	1 (1.7)	2 (3.4)	4 (10.5)	0.10
M41L	3 (5.0)	5 (8.5)	6 (15.8)	0.19
HIV Subtype				
A	3 (5.0)	2 (3.4)	-	
CRF01_AE	-	1 (1.7)	1 (2.6)	
CRF02_AG	39 (65.0)	38 (64.4)	27 (71.1)	
CRF09_cpx	-	1 (1.7)	-	
CRF11_cpx	2 (3.3)	1 (1.7)	1 (2.6)	
CRF13_cpx	-	-	1 (2.6)	
CRF18_cpx	-	1 (1.7)	1 (2.6)	
CRF22_01A1	3 (5.0)	4 (6.8)	1 (2.6)	
CRF37_cpx	2 (3.3)	-	1 (2.6)	
CRF43_02G	1 (1.7)	-	-	
D	1 (1.7)	-	2 (5.3)	
F2	1 (1.7)	3 (5.1)	6	
G	3 (5.0)	3 (5.1)	-	
URF*	5 (8.3)	5 (8.5)	3 (7.9)	

*Unique Recombinant Form

Ce projet au Cameroun a été réalisé grâce à des financements provenant d'Expertise France (anciennement GIP-ESTHER ; Ensemble pour un Solidarité Thérapeutique Hospitalière En Réseau) qui mettait en relation des hôpitaux français avec des hôpitaux au niveau central dans le PRL afin d'améliorer la prise en charge des personnes infectées par le VIH par l'offre de formations et le transfert de compétences. Au Cameroun les TARV sont disponibles depuis 2004, et gratuits depuis 2007. Afin d'élargir l'accès aux TARV, une politique de décentralisation des soins a été mise en place. Cette politique s'appuie sur les Centres de Traitement Agrées (CTA) dans les hôpitaux centraux et des Unités de Prise en Charge (UPEC) des Personnes vivant avec le VIH dans les hôpitaux de district, répartis sur l'ensemble du territoire. Dans le cadre des activités du GIP-ESTHER, les UPEC étant « parrainées » par des CTA dit « tuteurs » dans chaque région et appuyés par les équipes des centres hospitalo-universitaires français. Ce projet de tutorat dit de « première génération » a été mis en œuvre en 2008 par la Direction de la Lutte contre la Maladie assistée du GIP-ESTHER et d'autres partenaires du MINSANTE. Ce tutorat consistait en l'accompagnement des formations sanitaires de niveau inférieur par des formations sanitaires de niveau supérieur dans la mise en place des services efficaces et durables dans le domaine de la prise en charge globale des PVVIH. Il devait ainsi les préparer à long terme à acquérir leur autonomie pour pouvoir, à leur tour, accompagner d'autres formations sanitaires de niveau inférieur. Ce projet de «Décentralisation par le tutorat de la prise en charge du VIH/SIDA au Cameroun» a été mis en place de 2008 à 2012 et a permis le soutien de 13 CTA sur les 23 du pays. Ces 13 CTA ont mené des activités de tutorat vers 76 UPEC (sur les 106 prévues dans le projet et les 166 recensées au Cameroun). Un premier rapport intitulé « Evaluation du programme MINSANTE/ESTHER de décentralisation de la prise en charge globale des PVVIH au Cameroun par le tutorat de 1ère génération » avait été réalisé en 2014 et avait permis de mettre en évidence des problématiques structurelles et organisationnelles. Cependant, cette évaluation n'incluait pas de données de succès virologique ou de données centrées sur les patients mais visait essentiellement les structures. Ainsi, les données générées par la mise en place des études que nous avons menées ont permis d'apporter ces informations et de mettre en évidence un faible contrôle virologique des patients suivis en zone décentralisée au Cameroun ainsi qu'un très faible taux de rétention, s'aggravant au cours du temps. Ces données sont importantes afin que le programme national de lutte contre le SIDA puisse mettre en place des mesures adaptées.

De plus, la mesure de la CV de ces patients n'aurait pas pu être possible sans l'utilisation du DBS comme support de prélèvement. En effet, les infrastructures de laboratoire de ces structures périphériques ne permettent pas la réalisation de telle mesure dans les conditions actuelles. L'état des routes notamment pendant la saison des pluies et particulièrement dans la région Est du Cameroun ainsi que l'éloignement géographique important des centres d'un laboratoire capable de réaliser les mesures de CV sur plasmas,

rendent également l'utilisation et le transport des plasmas quasiment impossible ou tout du moins très onéreux en vue d'obtenir des résultats faibles de CV pour les patients.

Photos illustrant l'état des routes pour accéder à certains sites de prise en charge évalués

Photos illustrant l'état des laboratoires dans certains sites de prise en charge évalués

Au Vietnam, nous avons travaillé en collaboration avec le laboratoire de virologie du département VIH/SIDA du National Institute of Hygiene and Epidemiology de Hanoï, institut rattaché au Réseau International des Instituts Pasteur (https://www.pasteur.fr/fr/institut-pasteur/dans-le-monde/reseau-international-instituts-pasteur). Ce laboratoire est directement impliqué dans les activités de recherche et les activités connexes d'animation, de gestion et de formation dans le cadre du dépistage du VIH, de la surveillance, du suivi et de l'évaluation des programmes de prévention et de contrôle du VIH/sida à l'échelle nationale. Il fait partie du comité de surveillance du VIH/sida et du conseil d'administration par intérim du Programme national de ciblage sur le VIH/sida. VIH/SIDA pour les provinces du Nord du Vietnam. Le laboratoire de virologie du département VIH/SIDA est le laboratoire national de référence en matière de VIH. Les laboratoires de sérologie et de biologie moléculaire sont accrédités ISO 15189. Les tâches des laboratoires du VIH sont entre autres : Diagnostic du VIH, suivi des CD4 et de la CV, tests de résistance aux médicaments anti-VIH. Le laboratoire est en charge également du système d'assurance qualité externe au niveau sérologique et moléculaire dans le domaine du VIH en envoyant deux fois par an un panel à plus de 500 laboratoires dans tout le pays.

Comme énoncé dans l'introduction, ce laboratoire n'avait jamais réalisé de mesure de la CV sur DBS. Nous avons, dans un premier temps, mis en place deux études permettant de s'assurer de la qualité des résultats de CV sur DBS en prenant les résultats de CV obtenus sur plasma avec la même technique de RT-PCR comme mesure de référence (gold standard). Les techniques de mesure de la CV que nous avons évaluées sur DBS sont les techniques commerciales actuellement disponibles sur le marché au Vietnam.

Evaluation of Two Techniques for Viral Load Monitoring Using Dried Blood Spot in Routine Practice in Vietnam (French National Agency for AIDS and Hepatitis Research 12338). Open Forum Infect Dis. 2016 Jul 7;3(3):ofw142

Open Forum Infectious Diseases

MAJOR ARTICLE

Evaluation of Two Techniques for Viral Load Monitoring Using Dried Blood Spot in Routine Practice in Vietnam (French National Agency for AIDS and Hepatitis Research 12338)

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Background. Although it is the best method to detect early therapeutic failure, viral load (VL) monitoring is still not widely available in many resource-limited settings because of difficulties in specimen transfer, personnel shortage, and insufficient laboratory infrastructures. Dried blood spot (DBS) use, which was introduced in the latest World Health Organization recommendations, can overcome these difficulties. This evaluation aimed at validating VL measurement in DBS, in a laboratory without previous DBS experience and in routine testing conditions.

Methods. Human immunodeficiency virus (HIV)-infected adults were observed in a HIV care site in Hanoi, and each patient provided 2 DBS cards with whole blood spots and 2 plasma samples. Viral load was measured in DBS and in plasma using the COBAS Ampliprep/TaqMan and the Abbott RealTime assays. To correctly identify those with VL ≥ 1000 copies/mL, sensitivity and specificity were estimated.

Results. A total of 198 patients were enrolled. With the Roche technique, 51 plasma VL were ≥1000 copies/mL; among these, 28 presented a VL in DBS that was also ≥1000 copies/mL (sensitivity, 54.9; 95% confidence interval [CI], 40.3–68.9). On the other hand, all plasma VL < 1000 copies/mL were also <1000 copies/mL in DBS (specificity, 100; 95% CI, 97.5–100). With the Abbott technique, 45 plasma VL were ≥1000 copies/mL; among these, 42 VL in DBS were also ≥1000 copies/mL (sensitivity, 93.3%; 95% CI, 81.7–98.6); specificity was 94.8 (95% CI, 90.0–97.7).

Conclusions. The Abbott RealTime polymerase chain reaction assay provided adequate VL results in DBS, thus allowing DBS use for VL monitoring.

Keywords. HIV; Viral load; Dried Blood Spots (DBS); virological failure; sensitivity.

In recent years, the number of human immunodeficiency virus (HIV)-infected patients on antiretroviral therapy (ART) has dramatically increased [1]. Early detection of therapeutic failure is a major challenge for the management of HIV patients on ART, to optimize ART efficacy and prevent accumulation of resistance mutations that may compromise future treatment options [2, 3]. In its latest update, the World Health Organization (WHO) encouraged routine viral load (VL) monitoring of patients on ART to identify virological failure (VF) [4]. Efforts have been made to widen access to VL monitoring, although VL monitoring remains scarce in remote areas due to difficulties in specimen transfer, personnel shortages, and insufficient laboratory infrastructures [5].

To overcome these obstacles, one strategy involves point-of-care (POC) machines capable of performing VL measurement in field conditions where infrastructures and highly trained personnel are unavailable [6–8]. Field evaluations of these POC machines have been promising [9, 10], but larger scale validations are required. The advantages of POC machines are that they provide quick results to the patient and have low detection limits. However, equipping each care site may also pose problems due to costs and the need for maintenance and continuous reagent supplies.

A second strategy, introduced into the most recent WHO guidelines [4], makes use of dried blood spots (DBS) as a sampling tool, with the subsequent transfer of DBS to a central laboratory. Some studies have shown that DBS is a useful, efficient tool for monitoring VL in various settings [11, 12], offering a

Received 28 April 2016; accepted 27 June 2016.

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DOI: 10.1093/ofid/ofw142

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practical and easy means of broadening access to VL monitoring for patients observed in remote areas and in level I or II facilities [13]. At the care site level, DBS are easy to perform because they do not require skilled personnel or purchase of new equipment. They are easy to maintain and can be kept at ambient temperature for at least 2 weeks before freezing [14–17]. This capacity facilitates their transfer, which can be conducted through the existing postal network; the DBS are also not contagious. Moreover, at the central laboratory, the technique used for VL monitoring is the same as that used on plasma, and thus it does not require new machines. Dried blood spots also allow monitoring for HIV drug resistance [11, 12, 18]. Disadvantages of DBS are mainly the laboratory manual time imposed by the DBS technique and a higher detection threshold.

In Vietnam, the latest UNAIDS figures estimated the prevalence of HIV in adults as 0.5% [19]. Antiretroviral therapy coverage was estimated at 67.1% in 2013 [20]. Laboratories able to perform VL monitoring exist solely in large cities such as Hanoi and Ho Chi Minh City. Currently, patients observed in remote areas have no or extremely low access to VL monitoring.

Before implementing VL monitoring using DBS on a wide scale, we evaluated the capacity of molecular testing to identify those with a VL $\geq$ 1000 copies/mL in DBS compared with plasma, in routine testing conditions, using fully automatized polymerase chain reaction (PCR) platforms and manufacturer's DBS protocols from Roche and Abbott, which are currently available in Vietnam.

METHODS

Patients and Samples

The study protocol was approved by the Institutional Review Board from the Hanoi School of Public Health (Hanoi, Vietnam). Only participants providing written informed consent were enrolled, and random anonymous identification numbers were assigned to each patient to guarantee confidentiality.

Adults (>18 years old) observed at the Dong Da outpatient clinic in Hanoi were invited to participate in the study. To obtain a wide range of VL levels, 3 groups of patients were targeted: ART-naive patients, patients on ART for <6 months, and patients on ART for at least 6 months. Patients who consented to participate in the study provided a whole blood sample, and a short questionnaire recorded each participant's gender, age, ART group, and suspicion of treatment failure (based on clinical and immunological criteria). For each participant, the whole blood sample and information form were identified using a randomly attributed identification number. Whole blood samples were transferred the same day to the HIV/acquired immune deficiency syndrome (AIDS) molecular laboratory (National Reference HIV Molecular Laboratory) at the National Institute of Hygiene and Epidemiology (NIHE) in Hanoi.

At the HIV/AIDS molecular laboratory at NIHE, for each patient, a predefined volume (50 µL for use with the Abbott technique and 70 µL for use with the Roche technique) of whole blood was deposited using a calibrated pipette on 2 Munktell TFN cards on each of the 5 precut spots. The DBS cards were left to dry at ambient temperature for 3 hours and were then individually packed in ziplock bags with 2 desiccants. To reproduce the routine practice, DBS were kept for 2 weeks at ambient temperature (maximum delay between DBS collection and reception at the central laboratory) and then placed in a freezer at −20°C until VL measurement (until enough samples were collected to run a compete batch). After the making of the DBS cards, the remaining blood was centrifuged and 2 plasma samples of 2 mL were collected in cryotubes and stored at −80°C until VL measurement.

To ensure independent VL measurements from a same patient, blinded conditions were established by using preidentified stickers with randomly allocated identification numbers for DBS cards and for cryotubes. The correspondence list was created and stored by investigators at Institut Pasteur (Paris, France).

Laboratory Techniques

For each of the following 2 techniques (Roche and Abbott), VL measurements were independently performed in plasma and in DBS. Viral load results in DBS were then compared with the plasma VL (gold standard).

Viral Load Measurement on Plasma

Following manufacturers' protocol (COBAS AmpliPrep TaqMan HIV-1v 2.0 and Abbott Real-time HIV-1), the plasma input volume was 1.1 mL for Roche and 0.8 mL for Abbott. The nucleic acid extraction product was 65 µL for Roche and 70–80 µL for Abbott. For both techniques, the volume used for PCR was 50 µL.

Viral Load Measurement on Dried Blood Spot

In the Roche technique, the new free virus elution (FVE) method [21] was used. One spot of 70 µL was incubated in 1 mL calcium and magnesium-free phosphate-buffered saline ([PBS] 154 nM NaCl, 5.6 nM Na_2HPO_4, 1.1 nM KH_2PO_4, pH 7.4; Corning) in a tube at room temperature for 30 minutes. Using the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test v2.0 (Roche Molecular Systems, Branchburg, NJ), we directly extracted ribonucleic acid (RNA) in the tube containing the elution buffer and the spot specifying the DBS protocol (HI2DFSP96). According to the manufacturer's recommendation for this protocol, a correction factor (+0.3 log copies/mL) was added to each result on DBS [21, 22].

In the Abbott technique, following the manufacturer's protocol, 2 spots of 50 µL were placed in a tube with 1.7 mL Abbott Lysis Buffer (*m*Lysis Buffer LN2N77-01). After a 1-hour elution, RNA extraction was performed on m2000sp using 1 mL of the buffer. Amplification and detection were performed on m2000rt using the 1 mL DBS HIV-1 RNA protocol, specifying that the sample originated from DBS.

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Statistical Analysis

In accordance with WHO guidelines, VF was defined as a VL ≥ 1000 copies/mL [4]. To conduct a qualitative evaluation of the 2 techniques, sensitivity and specificity were estimated. The sensitivity was the proportion of patients with VL ≥ 1000 copies/mL in DBS among those with plasma VL ≥ 1000 copies/mL. The specificity was the proportion of patients with VL < 1000 copies/mL in DBS among those with plasma VL < 1000 copies/mL. Sensitivity and specificity were also estimated at the thresholds of 3000 and 5000 copies/mL. The concordance coefficient was the proportion of measurements, both above or both below the threshold for plasma and for DBS. Qualitative evaluations were conducted estimating correlations between plasma and DBS VL and through Bland-Altman analysis.

RESULTS

From July to October 2015, 198 patients were enrolled. Most patients were male (145 of 198), and the median age was 37 years. Regarding ART history, 19 (9.6%) were ART naive, 34 (17.2%) were on ART for <6 months, and the remaining 145 (73.2%) were on ART for ≥6 months; among these latter patients, 94 (64.8%) were suspected of therapeutic failure.

The median delay between blood sampling and VL measurement in DBS was 28 (interquartile range [IQR], 18–71) days for the Roche technique and 27 (IQR, 22–70) days for the Abbott technique ($P = .36$).

Plasma Viral Loads

With the Roche technique, 83 (41.9%) plasma samples were undetectable for HIV RNA (<20 copies/mL). In the remaining 115 samples, the median VL level was 601 (IQR, 115–33200) copies/mL. With the Abbott technique, 116 (58.6%) plasma samples were undetectable for HIV RNA (<40 copies/mL). In the remaining 82 samples, the median VL level was 1778 (IQR, 179–43 651) copies/mL. Plasma VL obtained with the 2 techniques were highly correlated ($\rho = .96$, $P < .001$).

At the threshold of 1000 copies/mL, agreement between the 2 techniques was observed in all but 6 VL measurements: these measurements were above 1000 copies/mL with the Roche technique (range, 1220–3250 copies/mL), but were below 1000 copies/mL with the Abbott technique, although above the lower detection limit (range, 80–814 copies/mL).

Evaluation of the Roche Technique

From a strictly qualitative point of view, the correlation between plasma and DBS VL was good ($\rho = .84$, $P < .001$) (Figure 1A), despite underestimation on DBS. This underestimation is also highlighted in the Bland-Altman analysis (mean difference, −0.35 log copies/mL) (Figure 2A).

Of 198 plasma VL, 51 (25.8%) were ≥1000 copies/mL. Among these, 28 VL results were also ≥1000 copies/mL in DBS (sensitivity, 54.9%; 95% confidence interval [CI], 40.3–68.9) (Table 1). Of the 147 plasma VL < 1000 copies/mL, all were also below this threshold in DBS (specificity, 100%; 95% CI, 97.5–100). The concordance coefficient was 88.4% (95% CI, 83.1–92.5).

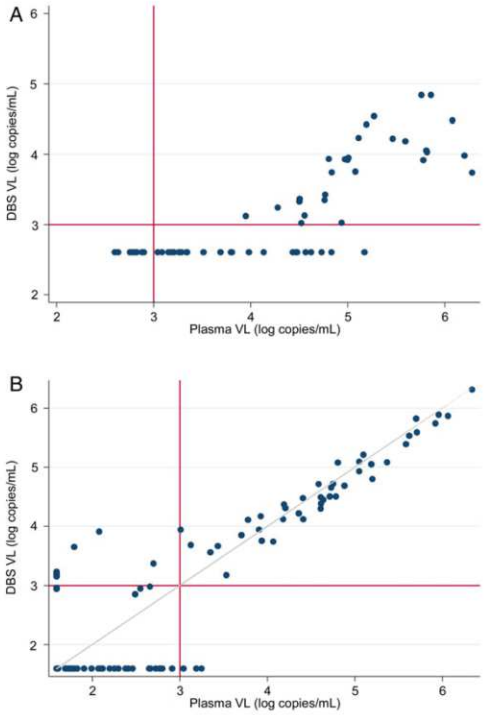

Figure 1. Correlation between viral load (VL) measurements on plasma and on dried blood spots (DBS). (A) Roche technique; (B) Abbott technique.

Although not significant, sensitivity at the threshold of 1000 copies/mL tended to be larger when the delay between DBS collection and VL measurement in DBS was <4 weeks, compared with ≥4 weeks (68.0% vs 42.3%, $P = .06$). However, specificity remained 100%, regardless of the delay. When stratifying analysis by ART duration (<6 or ≥6 months), sensitivity was not modified ($P = .40$).

When VF was defined at the threshold of 3000 and 5000 copies/mL, the sensitivity was 45.2% (95% CI, 29.8–61.3) and 47.5% (95% CI, 31.5–63.9), respectively. The specificity remained at 100%.

Evaluation of the Abbott Technique

A strong correlation was found between VL measured on plasma and on DBS ($\rho = .93$, $P < .001$) (Figure 1B). In the Bland-Altman analysis, the mean difference between DBS and plasma VL measurement was −0.03 log copies/mL (Figure 2B). Concordance was particularly good above the threshold of 3 log copies/mL.

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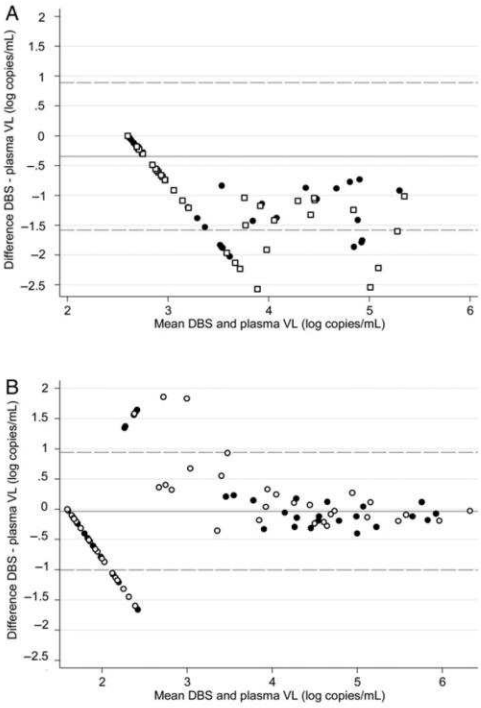

Figure 2. Bland-Altman analysis of agreement between dried blood spots (DBS) and plasma viral loads (VL). (A) Roche technique; (B) Abbott technique. The horizontal lines represent the mean (solid line) and the ± 1.96 standard deviations (dashed lines). Empty square: on antiretroviral therapy (ART) for <6 months; full circles: on ART for ≥6 months.

Of 198 plasma VL, 45 (22.7%) were ≥1000 copies/mL. Among these, 42 were also ≥1000 copies/mL in DBS (sensitivity, 93.3%; 95% CI, 81.7–98.6) (Table 2). Of the 153 plasma VL < 1000 copies/mL, 145 VL were also <1000 copies/mL in DBS (specificity, 94.8%; 95% CI, 90.0–97.7). The concordance coefficient between plasma and DBS results was 94.4% (95% CI, 90.3–97.2).

Three patients with a plasma VL ≥ 1000 copies/mL, ranging from 1096 to 1543 copies/mL, were undetectable when VL was measured in DBS. In these patients, the delay between blood collection and VL measurement in DBS were 17, 22, and 70 days; it was not significantly different from the delay observed in other patients with a plasma VL ≥ 1000 copies/mL ($P = .80$). Of note, with the Roche technique, these patients also presented intermediate plasma VL results (range, 1630–1920 copies/mL) and undetectable HIV RNA in DBS.

Eight patients with plasma VL < 1000 copies/mL presented a VL ≥ 1000 copies/mL in DBS. Four were on ART for more than 6 months and had undetectable plasma VL but moderately high VL in DBS (1427 to 1708 copies/mL). The other 4 were on ART for <6 months and had low plasma VL (1 with plasma VL < 40 copies/mL, otherwise 62 to 502 copies/mL); of note, these 4 patients had detectable plasma VL with the Roche technique. The corresponding VL in DBS ranged from 1480 to 8128 copies/mL.

Table 1. Sensitivity and Specificity of the Techniques on DBS Compared With Plasma, Roche Technique

Threshold-Defining Failure	VL > Threshold on Plasma	VL ≤ Threshold on Plasma
1000 copies/mL		
VL > threshold on DBS	28	0
VL ≤ threshold on DBS	23	147
Total	51	147
	Se, 54.9 (95% CI, 40.3–68.9)	Sp, 100 95% CI, 97.5–100)
3000 copies/mL		
VL > threshold on DBS	19	0
VL ≤ threshold on DBS	23	156
Total	42	156
	Se, 45.2 (95% CI, 29.8–61.3)	Sp, 100 (95% CI, 97.7–100)
5000 copies/mL		
VL > threshold on DBS	19	0
VL ≤ threshold on DBS	21	158
Total	40	158
	Se, 47.5 (95% CI, 31.5–63.8)	Sp, 100 (95% CI, 97.7–100)

Abbreviations: CI, confidence interval; DBS, dried blood spots; Se, sensitivity; Sp, specificity; VL, viral load.

Table 2. Sensitivity and Specificity of the Techniques on DBS Compared With Plasma, Abbott Technique

Threshold-defining failure	Plasma VL > Threshold	Plasma VL ≤ Threshold
1000 copies/mL		
VL > threshold in DBS	42	8
VL ≤ threshold in DBS	3	145
Total	45	153
	Se, 93.3 (95% CI, 81.7–98.6)	Sp, 94.8 (95% CI, 90.0–97.7)
3000 copies/mL		
VL > threshold in DBS	37	6
VL ≤ threshold in DBS	1	154
Total	38	160
	Se, 97.4 (95% CI, 86.2–99.9)	Sp, 96.3 (95% CI, 92.0–98.6)
5000 copies/mL		
VL > threshold in DBS	37	2
VL ≤ threshold in DBS	0	159
Total	37	161
	Se, 100 (95% CI, 90.5–100)	Sp, 98.8 (95% CI, 95.6–99.8)

Abbreviations: CI, confidence interval; DBS, dried blood spots; Se, sensitivity; Sp, specificity; VL, viral load.

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At the threshold of 1000 copies/mL, sensitivity and specificity were not different when VL in DBS was measured <4 or ≥4 weeks after DBS collection ($P = .68$ and $P = .63$, respectively). When stratifying analysis by ART duration (<6 or ≥6 months), sensitivity was not modified ($P = .99$) and specificity tended to be improved (88.2% vs 96.6%, $P = .07$).

At the threshold of 3000 copies/mL, sensitivity was 97.4% (95% CI, 86.2–99.9) and specificity was 96.3% (95% CI, 92.0–98.6). At the threshold of 5000 copies/mL, sensitivity was 100% (95% CI, 90.5–100) and specificity was 98.8% (95% CI, 95.6–99.8).

DISCUSSION

From a clinical viewpoint, we conducted a qualitative evaluation because decisions regarding ART management are taken essentially based on VL measurements above or below a specific threshold. At the threshold of 1000 copies/mL, sensitivity (ability to identify patients in VF) was high with the Abbott technique (93.3%) and resembled findings of previous reports [18, 23]. On the other hand, the sensitivity was low (54.9%) with the Roche technique.

The sensitivity of the Abbott technique was high, and only 3 patients with a plasma VL result ≥1000 copies/mL were undetectable in DBS. It is of interest to note that, for these 3 patients, the same result occurred with the Roche technique. The delay between DBS collection and VL measurement did not seem to account for this result, because it was not different from the delay observed in the other patients. In these 3 patients, the plasma VL level was low (1096–1776 copies/mL). Degradation of RNA may have occurred because of bad conservation conditions of the DBS cards and thus may explain this result. An incapacity to identify patients failing on ART is a serious problem. Nevertheless, such patients would likely be identified as failing at their next virological evaluation, even though it means that identifying this failure would be delayed.

The sensitivity observed with the Roche technique was much lower than in previous evaluations [21, 24]. The new elution (FVE) protocol used in the present study has been the subject of a single previous evaluation [21] in which the authors stated that PBS use during the elution phase offers the advantage of reducing the overquantification of HIV VL in DBS by limiting proviral deoxyribonucleic acid (DNA) amplification, thus explaining the good specificity. The authors also acknowledged that it may also underestimate HIV RNA quantification in DBS. This result could explain the reduced sensitivity observed. The present evaluation was conducted with field-collected samples, in routine practice and in a laboratory not dedicated to research. Such factors could also explain the difference observed. In the evaluation by Andreotti et al [24], the sensitivity was also higher than that observed in the present study. However, extraction was performed with the NucliSENS miniMAG system (Biomerieux, Marcy l'Etoile, France), because their first attempt with the Roche COBAS TaqMan assay led to a significant underestimation. Despite this, VL on DBS was still underestimating plasma VL. The lower sensitivity we observed could also be explained by the use of Munktell TFN cards, whereas the other 2 studies used Whatman 903 DBS cards [25].

The difference in performance between the 2 techniques was unexpected. It should not be explained by condition of DBS conservation, because they were all maintained the same way. Furthermore, it could not be explained by the duration of conservation of the DBS cards, because the delay of VL measurement on DBS did not differ between the 2 techniques. Of note, sensitivity tended to decrease with the Roche technique when the delay between DBS collection and VL measurements on DBS was more than 4 weeks. However, even when this delay was less than 4 weeks, the sensitivity was only 68%. We found no evidence of an impact of this delay for the Abbott technique. The 2 techniques also differed in terms of quantity of blood used (1 spot of 70 µL for the Roche technique, 2 spots of 50 µL each for the Abbott technique) and in terms of elution procedure, which could potentially explain the sensitivity difference between the techniques.

Specificity evaluated the ability to identify patients with VL < 1000 copies/mL. The specificity was 100% using the Roche technique and slightly lower using the Abbott technique (94.8%).

With the Abbott technique, lack of detection of the virological success (VL in DBS ≥ 1000 copies/mL, whereas plasma VL < 1000 copies/mL) was likely due to proviral DNA and intracellular RNA contaminations [26, 27]. Eight patients with plasma VL < 1000 copies/mL presented VL ≥ 1000 copies/mL in DBS. It is interesting to note that 4 of these patients were on ART for <6 months. These patients may still present a large viral reservoir, although the circulating virus was reduced by ART, and the VL result in DBS could be due to amplification of proviral DNA. In routine practice, only patients with at least 6 months on ART would be evaluated, thus reducing the risk of false identification of failure. It must also be noted that, following current WHO guidelines [1] applied in Vietnam, patients with a VL ≥ 1000 copies/mL undergo counseling to improve ART adherence; and a new VL measurement at least 1 month later is conducted. Thus, the probability of having 2 consecutive (separated by 1 to 3 months with strengthening of adherence) false-positive VLs (VL ≥ 1000 copies/mL) is low, making a small default in specificity less worrisome, despite a small additional cost because of unnecessary VL retesting of a small proportion of patients.

For the Roche technique, Wu et al [21] suggest that the use of PBS in the elution protocol prevents proviral DNA amplification.

A systematic review of the use of DBS for VL monitoring showed that VL results on DBS, apart from the VL technique

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itself, depend on punch size, method for punching, elution buffer used, and sample volume after extraction [28]. To limit the impact of these interventions, procedures were standardized and followed manufacturers' recommendations. We used cards with precut spots to reduce the time needed to cut the spots and the risk of contamination with the use of a punch. Moreover, we minimized the disadvantages of the manual time imposed by the DBS technique by using as many automatized procedures as possible.

This study is one of the few studies conducted under "real-life" conditions. We sought to reproduce routine conditions using field-collected samples and to reproduce the delay between DBS collection and VL measurement, by maintaining the DBS cards at ambient temperature for 2 weeks before freezing. Therefore, all procedures used in this study can be implemented on a larger scale.

It is now recognized that use of DBS is the most feasible way to provide VL monitoring to patients observed in decentralized areas [29–31]. At the laboratory level, manual laboratory time can be greatly reduced with new elution protocols and automatization, but manufacturers have to integrate official guidelines for VL measurements using DBS to their manuals. It is important to note that machines used are those used for VL measurement on plasma and thus new equipment is not required. Moreover, DBS also allow monitoring HIV drug resistance in patients in VF [11, 12, 18].

CONCLUSIONS

Following current guidelines, clinical decisions regarding ART maintenance or modification were made based on VL $\geq$ 1000 copies/mL, rendering sensitivity as the most important performance criterion. High sensitivity is important to identify those patients for whom ART is failing. In our setting, the use of Abbott technique for VL measurement in DBS performed well. Moreover, when we followed the WHO suggestions for VL measurement on DBS and adopted the threshold defining failure as 3000 or 5000 copies/mL, the sensitivity and specificity of the Abbott technique reached approximately 100%.

In Vietnam, VL monitoring can now be implemented using the Abbott platform, centralizing the DBS for VL measurement at the molecular laboratory of NIHE.

Acknowledgments

We thank all of the patients who kindly agreed to participate in this study, the medical staff, and the national health authorities. We also thank Dr. Frédérique Guiroy (French National Agency for AIDS and Hepatitis Research [ANRS]) for valuable support.

Financial support. This work was supported by the Agence Nationale de Recherches sur le SIDA and Hépatites Virales (Paris, France). Abbott and Roche companies supported this study by providing viral load test kits.

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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Cette étude, appelée MOVIDA 1, a été permise par l'obtention d'un contrat d'initiation de l'ANRS (ANRS 12338) et la mise à disposition gratuite de la part des deux industriels (Roche et Abbott) de l'ensemble des kits de CV nécessaires à l'étude ainsi que de programmes de formation sur la mesure de la CV sur DBS sur chacune des techniques (chaque industriel s'occupant de la formation de l'équipe sur sa propre technique).

A la lumière des résultats obtenus, la qualité des résultats rendus par l'équipe de virologie via la qualité des courbes de corrélation obtenues, a été validé. Nous avons opté pour la technique Abbott dans le cadre de la cohorte de patients issus des zones décentralisées du Nord du Vietnam (projet MOVIDA 2). Cette technique remplit les recommandations de performances minimales demandées par l'OMS à savoir au minimum 85% de sensibilité et de spécificité (4).

Cependant, l'absence de marquage CE pouvait représenter un obstacle pour l'utilisation à large échelle de telles techniques car les recommandations d'utilisation des kits sur DBS doivent être validées par l'industriel et leurs performances par les autorités compétentes internationalement reconnues. Abbott a déposé et obtenu le marquage CE pour une nouvelle technique de mesure de la CV sur DBS. Ainsi, nous avons procédé à une nouvelle évaluation de cette nouvelle technique selon les mêmes modalités que pour l'étude précédente. Cette étude a été appelée « MOVIDA 1 bis ».

First field evaluation of the optimized CE marked Abbott protocol for HIV RNA testing on dried blood spot in a routine clinical setting in Vietnam. PLoS One. 2018 Feb 9;13(2):e0191920

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RESEARCH ARTICLE

First field evaluation of the optimized CE marked Abbott protocol for HIV RNA testing on dried blood spot in a routine clinical setting in Vietnam

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Citation: Taieb F, Tran Hong T, Ho HT, Nguyen Thanh B, Pham Phuong T, Viet Ta D, et al. (2018) First field evaluation of the optimized CE marked Abbott protocol for HIV RNA testing on dried blood spot in a routine clinical setting in Vietnam. PLoS ONE 13(2): e0191920. https://doi.org/10.1371/journal.pone.0191920

Editor: Paul Sandstrom, Public Health Agency of Canada, CANADA

Received: June 29, 2017

Accepted: January 12, 2018

Published: February 9, 2018

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Data Availability Statement: All relevant data are available from Open Science Framework at the following URL: https://osf.io/rzfv2/.

Funding: This work was funded by Abbott company. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: This work was funded by Abbott Company. This does not alter our

Abstract

Background

Viral load (VL) monitoring of HIV-infected patients in decentralized areas is limited due to logistic constraints. Dried Blood Spots (DBS) offer the opportunity to collect samples in remote area which can be easily transferred and tested at a central laboratory. The MOVIDA (Monitoring Of Viral load In Decentralized Area) project evaluated the performance of VL measurements on DBS using the new CE marked optimized Abbott protocol.

Methods

HIV-1 infected adults from three outpatient clinics in Hanoi (Vietnam) were enrolled into the study between 1 March and 13 April 2017. VL was measured on DBS using the optimized protocol provided by the manufacturer and compared to plasma VL as reference method on the Abbott m2000rt RealTime HIV-1 platform. Sensitivity was defined as the ability for DBS samples to correctly identify VL failure at the threshold of 1000 copies/mL of plasma, while specificity represented the ability to identify patients with a plasma HIV-RNA VL of <1000 copies/mL.

Results

A total of 203 patients were enrolled in the study, of which 152 (75%) were male. Median age was 38 [inter quartile range: 34–43] years. Of these patients, 37 were untreated, 38 on ART for <6 months and 117 were on ART for ≥6 months. A strong correlation between VL

adherence to PLOS ONE policies on sharing data and materials.

results in plasma and from DBS was observed (ρ = 0.95; p<0.001). Plasma VL was ≥1000 copies/mL in 71 patients. The sensitivity of DBS was 90.1% (95% confidence interval [CI]: 80.7–95.9) and the specificity was 96.2% (95% CI: 91.4–98.8).

Conclusions

The new optimized Abbott DBS protocol performed well in this study, meeting the WHO performance criteria for the use of DBS for HIV VL monitoring. Scaling up VL monitoring using DBS can be used to reach the last 90 in the UNAIDS targets of 90-90-90 to help end the AIDS epidemics. However, sensitivity remains the main challenge for manufacturers to prevent maintaining patients in virological failure on inefficient ART.

Introduction

In 2014, the UNAIDS launched their 90-90-90 treatment targets to end the AIDS epidemics [1].This means that by 2020, 90% of all people living with HIV should know their HIV status, 90% of all people with diagnosed HIV infection should receive sustained antiretroviral therapy (ART), and 90% of all people receiving antiretroviral therapy should have viral suppression.

Virological monitoring is crucial for patients on ART to accurately detect virological failure, and to modify ART in a timely manner. Clinical and immunological criteria have been shown to poorly predict virological failure [2–4]. The World Health Organization (WHO) currently recommends viral load (VL) monitoring at 6 months and 12 months after ART initiation and thereafter annually [5]. While VL monitoring is the standard of care in developed countries, its availability remains limited in many settings, often constrained by cost, technologies, human resources, etc. [6]. In remote areas, distance from sufficiently equipped laboratories able to perform VL measurements and sometimes availability of electricity further limit the use of VL.

To overcome the difficulties of VL monitoring in remote areas, several strategies have recently emerged to complement the existing approaches. Point-of-care (POC) devices have been developed, which allow rapid, on-site VL measurements [7]. POC devices show encouraging results [8, 9], but still require laboratory capacities. Dried blood spots (DBS) using venous or capillary whole blood has been included in the latest WHO recommendations [5]. However, the ability to diagnose virological failure using DBS still needs to be validated before being scaled up. Several studies conducting such evaluation have been published [10–13], with variable results.

Abbott Molecular has developed a semi-automated two-spot HIV-1 DBS protocol which has been used in a number of clinical research studies [10, 11, 13–16]. A comprehensive review of studies evaluating and comparing the performance of DBS samples to plasma samples for HIV VL quantification demonstrated that DBS assays can achieve sensitivity and specificity above 90%. To increase ease-of-use and to improve the assay performance, a one-spot DBS protocol for the Abbott RealTime HIV-1 assay was developed, this protocol was CE marked in 2016 [17].

The current study aimed to evaluate the sensitivity and specificity of the CE marked optimized DBS protocol in identifying patients in virological failure, defined by a VL above the threshold of 1000 copies/mL, in routine clinical settings using a laboratory in Hanoi, Vietnam, as compared to VL in plasma samples.

Methods

HIV-1 infected adults from three outpatient clinics (OPCs) in Hanoi (Vietnam) were invited to participate in the study. We anticipated that most patients on ART for≥6 months would exhibit low VL [18–20], patients on ART for <6 months would essentially present intermediate VL, while pre-ART patients would present high VL. Thus, in order to obtain a wide range of VL levels, pre-ART patients, patients on ART for <6 months and patients on ART for ≥6 months were included in the study.

Patients were given detailed information (oral and written) about the study, after which they decided to take part or not in the study. Patients who provided written informed consent were enrolled and were randomly assigned an identification number. A case-report form was completed by the clinician recording the gender and age of the patient and ART history. A whole blood sample was drawn from venipuncture, collected in EDTA tube and transferred in a cool box the same day (within 3 hours following sampling) to the HIV/AIDS molecular laboratory of the National Institute of Hygiene and Epidemiology (NIHE) in Hanoi (Vietnam) where plasma and DBS samples were processed, and VL measurements were performed.

At the HIV/AIDS molecular laboratory, 70μl of fresh whole blood were dropped on each of the 5 one-half-inch (12 millimeter) circles of two perforated Munktell TFN paper cards (DBS) using a calibrated pipette. DBS were then left to dry at ambient temperature for 3 hours before being packed individually in ziplock bags with two desiccants. DBS cards were maintained at ambient temperature for 2 weeks, after which they were frozen at -20˚C following the WHO guidelines. This was done in order to mimic real life settings where delays between DBS collection, reception at a central laboratory and VL measurement often occur. The remaining whole blood was centrifuged at 2000–2500 rpm for 10 minutes and two plasma samples of 2mL were collected in cryotubes and frozen at -80˚C until VL was measured.

To ensure VL measurements from plasma and from DBS for the same patient were blinded, pre-identified stickers with randomly allocated identification numbers for EDTA tubes, DBS cards and plasma cryotubes were used. The correspondence list was created and stored by investigators at Institut Pasteur (Paris, France).

VL measurement on plasma

VL was quantified using the Abbott real-time HIV-1 PCR with 0.8 mL of plasma in the m2000rt system following the manufacturer's recommendations. After automated nucleic acid extraction, a volume of 50 μL was used for PCR. The lower detection limit was 40 copies/mL.

VL measurement on DBS

NIHE used the Open Mode HIV-1 RNA Quantitative DBS protocol file for HIV VL testing. Following the manufacturer's recommendations, one single DBS spot of 70μL per patient was placed into a tube with 1.3 mL of mDBS buffer (List No. 09N02-001). The tubes were mixed by swirling and incubated for 30 minutes in a heating block (Benchmark Scientific) at 55˚C. Samples were directly loaded on the m2000sp platform without additional pre-analytical manipulation. Open mode 1.0 mL HIV-1 RNA DBS IUO TT version 11 protocol was used for extraction and amplification. The Abbott HIV-1 RealTime assay uses automated extraction of viral nucleic acid on the m2000sp using purification reagents and purification process specific for RNA. The lower detection limit of the assay indicated by the manufacturer was 839 copies/mL.

Statistical analysis

Plasma VL results were considered as the gold standard. Plasma VL measurements below the threshold were arbitrarily attributed the value 20 copies/mL. DBS VL results below the detection limit were also attributed the value 20 copies/mL.

Correlation between log-transformed plasma and DBS VL measurements was estimated. A bland-Altman analysis was conducted to evaluate the concordance between the two methods.

Sensitivity and specificity with 95% confidence intervals (CI) of DBS to identify virological failure at the threshold of 1000 copies/mL were measured. Sensitivity was estimated as the proportion of plasma VL $\geq$1000 copies/mL for which DBS VL was also $\geq$1000 copies/mL. Specificity was estimated as the proportion of plasma VL $<$1000 copies/mL for which DBS VL was also $<$1000 copies/mL. The Kappa coefficient was used to measure agreement between plasma and DBS measurements.

Ethical consideration

The study protocol was approved by the Institutional Review Board from the Hanoi School of Public Health (Hanoi, Vietnam) (decision number 333/2016/YTCC-HD3), and the Institutional Review Board from Institut Pasteur (decision number 2016–05 IRB/1). Authorization for data processing has been obtained from French legal authority (Commission Nationale Informatique et Liberté, decision number DR-2017-046). Only participants providing written informed consent were enrolled, and random anonymous identification numbers were assigned to each patient to guarantee confidentiality.

Results

A total of 203 patients were enrolled in the study between March 1st and April 13th 2017 in three OPCs in Hanoi. Of these patients, 152 (74.9%) were male and the median (inter quartile range (IQR)) age was 38 (34–43) years.

The plasma VL was below the detection limit in 104 (51.2%) patients. In patients with detectable plasma VL, the median (IQR) level was 4.01 (2.91–5.00) log copies/mL. DBS VL measurements were performed after a median (IQR) delay of conservation of 19 (12–26) days, and this delay never exceeded 27 days. DBS VL was below the detection limit in 132 (65.0%) patients. In patients with detectable DBS VL, the median (IQR) was 4.29 (3.79–5.21) log copies/mL. The correlation between plasma and DBS VL measurements was 0.95 ($p<0.001$) (Fig 1). The bland-Altman analysis showed overall good concordance between plasma and DBS VL measurements (Fig 2). The aligned dots at the bottom left of the graph were due to undetectable DBS VL measurements.

Out of the 203 patients, 71 (35.0%) had plasma VL above the threshold of 1000 copies/mL. Of these patients, 64 also had DBS VL above the threshold of 1000 copies/mL. This meant a sensitivity of 90.1% (95% CI: 80.7–95.9) (Tables 1 and 2). Downward classification was observed in 7 patients (plasma VL $\geq$1000 copies/mL but DBS VL $<$1000 copies/mL). In these 7 patients, the plasma VL ranged from 1023 copies/mL to 2570 copies/mL, and was $<$1200 copies/mL in 4 patients (S1 Table).

Of the 203 patients, 132 (65.0%) had plasma VL below the threshold of 1000 copies/mL. Of these patients, 127 also had a DBS VL below this threshold. This meant a specificity of 96.2% (95% CI: 91.4–98.8) (Tables 1 and 2). Upward misclassification was observed in 5 patients (S2 Table). In 4 of these patients, plasma VL was detected at a low level (range: 166 to 479 copies/mL) while it was above 1000 copies/mL in DBS (range: 1102 to 4934 copies/mL). Only one patient had an undetectable plasma VL and a DBS above 1000 copies/mL (1478 copies/mL).

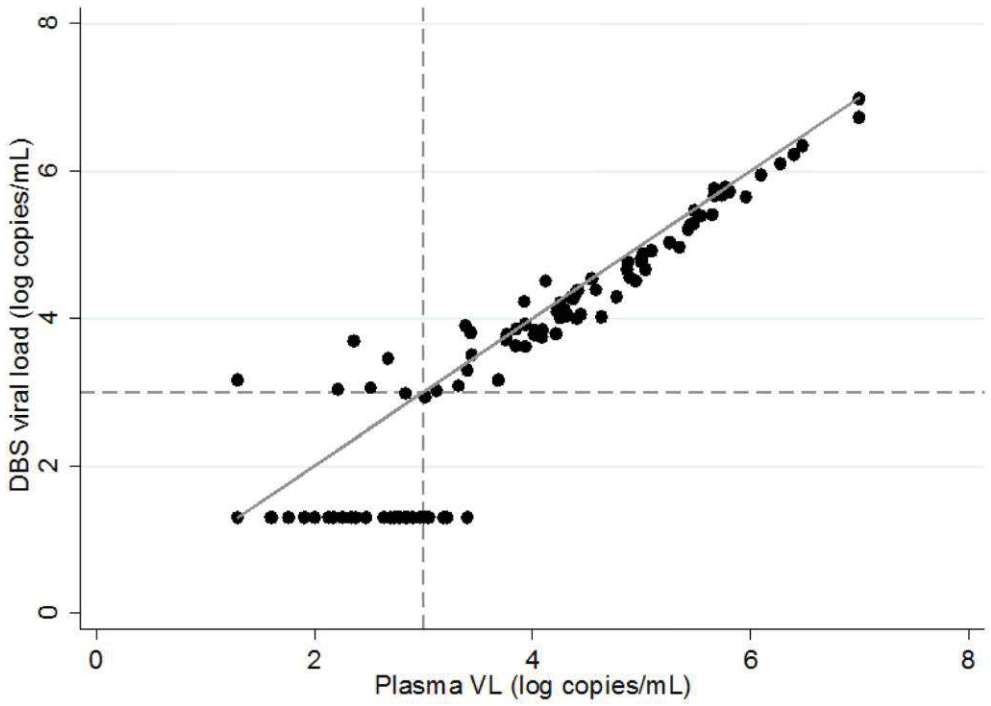

Fig 1. Correlation between plasma and DBS viral load measurements. The dashed line represents the threshold of 3 log copies/mL defining virological failure. The solid line represents the line of slope 1 displaying perfect concordance. Correlation: 0.95 ($p<0.001$).

https://doi.org/10.1371/journal.pone.0191920.g001

At the threshold of 1000 copies/mL, concordance between plasma and VL results was 94.1% (95% CI: 89.9–96.9). The Kappa coefficient was 86.9% showing excellent concordance between the DBS and plasma VL measurements.

Increasing the DBS threshold to 3000 copies/mL, 60 of the 71 patients with plasma VL above 1000 copies/mL were in virological failure. This corresponded to a sensitivity of 84.5% (95% CI: 74.0–92.0) (Table 3). On the other hand, 131 out of 132 patients with plasma VL below 1000 copies/mL were considered in virological success. This corresponded to a specificity of 99.2% (95% CI: 95.9–100). Further increasing the DBS threshold to 5000 copies/mL decreased the sensitivity while the specificity reached 100% (Table 3). Lowering the DBS threshold to 839 copies/mL (limit of detection provided by manufacturer), sensitivity was 91.5% (95% CI: 82.5–96.8) and specificity was 95.5% (95% CI: 90.3–98.3) (Table 3).

Impact of the duration on ART

To evaluate the impact of the ART duration on the DBS VL measurement, a stratified analysis was conducted considering patients on ART for <6 months on the one hand, and patients on

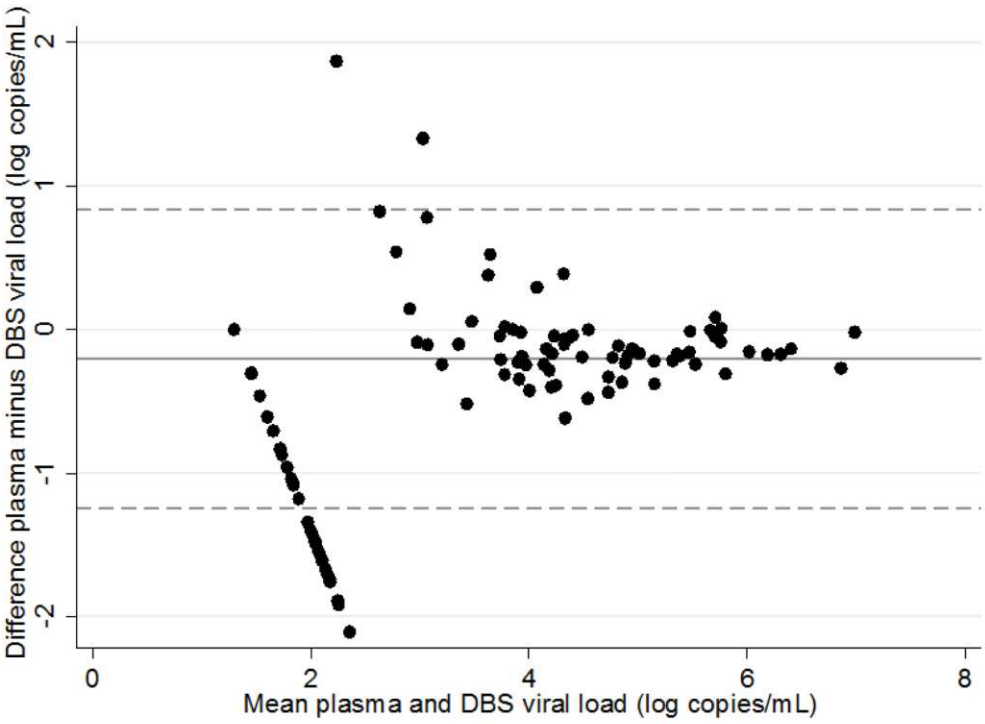

Fig 2. Bland-Altman analysis to evaluate concordance between plasma and DBS viral load measurements. The solid line is the mean difference between plasma and DBS viral load measurements and the dashed line is 95% confidence interval of the difference.

https://doi.org/10.1371/journal.pone.0191920.g002

ART for ≥6 months on the other hand. The ART duration could impact the quantity of blood cells harboring HIV and hence impact the specificity due to amplification of proviral DNA and/or intracellular RNA.

Restricting the analysis to the 39 patients on ART for <6 months, only 8 patients had a plasma VL ≥1000 copies/mL of whom 7 also had a DBS VL ≥1000 copies/mL. This

Table 1. Sensitivity and specificity of viral load measurements on DBS compared to plasma at the threshold of 1000 copies/mL.

	Plasma VL ≥1000 copies/mL	Plasma VL <1000 copies/mL	Total
DBS VL ≥1000 copies/mL	64 (90.1%)	5	69
DBS VL <1000 copies/mL	7	127 (96.2%)	134
Total	71	132	203

VL: viral load; DBS: dried blood spot

https://doi.org/10.1371/journal.pone.0191920.t001

Table 2. Distribution of plasma and DBS VL measurements.

	Plasma VL Category (copies/mL), N (%)				
DBS VL Category (copies/mL), N (%)	<1000	1000–2999	3000–4999	≥5000	Total
<1000	127 (96.2)	7 (53.9)[a]	-	-	134
1000–2999	4 (3.0)[b]	3 (23.1)	1 (100)	-	8
3000–4999	1 (0.8)[b]	1 (7.7)	-	2 (3.5)	4
≥5000	-	2 (15.4)	-	55 (96.5)	57
Total	132	13	1	57	203

VL: viral load; DBS: dried blood spot

[a] downward misclassification

[b] upward misclassification

https://doi.org/10.1371/journal.pone.0191920.t002

corresponded to a sensitivity of 87.5% (95% CI: 47.3–99.7). The 31 other patients had a plasma VL <1000 copies/mL, of whom 27 had also a DBS VL <1000 copies mL. This corresponded to a specificity of 87.1% (95% CI: 70.1–96.4).

Considering the 124 patients on ART for ≥6 months, 27 had a plasma VL ≥1000 copies/mL of which 23 also had a DBS VL ≥1000 copies/mL. This corresponded to a sensitivity of 85.2% (95% CI: 66.3–95.6). The remaining 97 patients had a plasma VL <1000 copies/mL of whom 96 also had a DBS VL <1000 copies/mL. This corresponded to a specificity of 99.0% (95% CI: 94.3–100.0).

Specificity was significantly better in patients on ART for ≥6 months than in those on ART for <6 months ($p = 0.012$). Sensitivity however did not differ by ART duration ($p = 0.99$).

Impact of the delay between DBS collection and VL measurements on DBS

DBS were kept at ambient temperature for two weeks and then frozen at -20˚C until VL measurements were performed. To investigate the impact of storage conditions on DBS VL measurements, we performed an analysis stratified on non- frozen DBS on one hand, and on frozen DBS on the other hand, as freezing was thought to impact the sensitivity.

Considering the 77 patients for whom DBS were not frozen, 25 had a plasma VL ≥1000 copies/mL. Of these, 23 also had a DBS VL ≥1000 copies/mL. This meant a sensitivity of 92.0% (95% CI: 74.0–99.0). The remaining 52 patients had a plasma VL and a DBS VL <1000 copies/mL. This meant a specificity of 100% (95% CI: 93.2–100).

Considering the 126 patients for whom DBS were frozen, 46 had a plasma VL ≥1000 copies/mL. Of these, 41 had a DBS VL that was also ≥1000 copies/mL. This meant a sensitivity of 89.1% (95% CI: 76.4–96.4). The remaining 80 patients had a plasma VL <1000 copies/mL. Of

Table 3. Sensitivity, specificity, misclassification, and Kappa coefficient by DBS testing and viral load thresholds on determining virological failure compared to plasma.

VL Threshold (copies/mL) Plasma: DBS	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Upward misclassification (%) (95% CI)	Downward misclassification (%) (95% CI)	Kappa coefficient (95% CI)
1000: TND	95.8 (88.1–99.1)	63.6 (54.8–71.8)	36.3 (28.2–45.2)	4.2 (0.9–11.9)	51.8 (41.4–62.2)
1000 : 839[a]	91.5 (82.5–96.8)	95.5 (90.3–98.3)	4.5 (1.7–9.6)	8.5 (3.2–1.7)	87.0 (79.9–94.1)
1000: 1000	90.1 (80.7–95.9)	96.2 (91.4–98.8)	3.8 (1.2–8.6)	9.9 (4.1–19.3)	86.9 (79.7–94.1)
1000: 3000	84.5 (74.0–92.0)	99.2 (95.9–100)	0.8 (0–4.1)	15.5 (8.0–26.0)	86.6 (79.2–93.9)
1000: 5000	80.3 (69.1–88.8)	100 (97.2–100)	0 (0–2.8)	19.7 (11.2–30.8)	84.1 (76.2–92.0)

VL: viral load; DBS: dried blood spot; CI: confidence interval; TND: target not detected[a] 839 copies/mL is the threshold provided by the manufacturer

https://doi.org/10.1371/journal.pone.0191920.t003

these, 75 also had a DBS VL <1000 copies/mL. This meant a specificity of 93.8% (95% CI: 86.0–97.9).

Neither sensitivity (p = 0.56) nor specificity (p = 0.08) differed significantly based on the freezing status of the DBS.

Discussion

This is the first field evaluation of the new CE marked optimized protocol developed by Abbott for VL quantification on DBS in routine clinical settings, in the laboratory responsible for VL monitoring in patients from the provinces of Northern Vietnam.

From a quantitative point of view, a very strong correlation was observed between plasma and DBS VL measurements. The estimated bias (mean difference between plasma and DBS VL measurements) was only 0.2 log copies/mL.

From a qualitative point of view, the capacity to detect virological failure at the threshold of 1000 copies/mL when using DBS was investigated through sensitivity and specificity which were 90.1% (95% CI: 80.7–95.9) and 96.2% (95% CI: 91.4–98.8), respectively. These results were comparable to those previously obtained when using venous whole blood collected on EDTA tubes, but using the previous two-spot Abbott DBS protocol [10, 11, 13, 15, 16].

With the new one-spot DBS protocol evaluated in the current study, sensitivity was 90.1% due to downward misclassification in 7 patients who presented DBS VL <1000 copies/mL. Of these 7 patients, DBS VL was detected in 4 (3 were below the detection limit of 839 copies/mL and 1 was 854 copies/mL) while their plasma VL was above the threshold of 1000 copies/mL, but the plasma VL level was modest as it ranged from 1043 to 1459 copies/mL. In the 3 remaining patients, DBS VL was not detected while their plasma VL was above the threshold of 1000 copies/mL (range: 1148 to 2570 copies/mL). Several explanations can be contemplated regarding these discrepancies. Firstly, inter-variability linked to the technique can't be ruled out, especially for the samples which were detected on DBS, even if the Abbott technique has been described as having a low variability (<10% of variability) at low copy level [21]. Secondly, downward misclassification could also be explained by the deterioration of RNA in the DBS due to the freezing at -20˚C after 15 days as recommended for HIV drug resistance genotyping [22]. Storage at -20˚C, when using the Roche assay, has been suggested to deteriorate VL results [11]; the manufacturer now recommends not to freeze or refrigerate samples for extended period of time when using free virus elution (FVE) protocol. However, we did not find an impact of the storage at -20˚C, a finding consistent with other studies [14]. Moreover, storage at -20˚C was an alternative mentioned by the manufacturer (Abbott). It must be noted however that the DBS samples were not subject to repeated freeze-thaw cycles. Thirdly, the whole blood volume and the elution process could explain the discrepancies between plasma and DBS VL results [21, 23]. In the optimized DBS protocol, one 70 μL spot (instead of two 50 μL spots in the previous protocol) is diluted into 1.3 mL of lysis buffer. On the other hand, 0.8 mL of plasma is used for the plasma technique. It could also be linked to the use of Munktell TFN cards that possibly have a lower sensitivity for HIV RNA testing by comparison to the Whatman 903 filter papers [24].

Specificity of the DBS protocol was 96.2% and upward misclassification was observed in 5 patients who presented DBS VL above 1000 copies/mL while the plasma VL was below this threshold. In 4 of these patients, plasma VL were detected but at a low level (range: 166 to 479 copies/mL) while DBS VL ranged from 1102 to 4934 copies/mL. In the remaining patient, plasma VL was <40 copies/mL and the corresponding DBS VL was 1478 copies/mL. Importantly, patients with a VL under the threshold of 1000 copies/mL were classified as being in virological success whether or not VL is detected. Of these 5 upwardly misclassified patients, 4

would have received a therapeutic intervention (strengthening of ART adherence and ART modification or intensification) following European or American guidelines due to the viral replication, even if it is at a low level to prevent therapeutic failure and HIV drug resistance acquisition [25–27]. Thus, the impact of the DBS results would have been beneficial for 4 of these 5 patients.

Upward misclassification could be due to amplification of proviral DNA and intracellular RNA contaminations [28, 29] especially when HIV DNA reservoir is high [30]. It must be noted that out of the 5 patients with upward misclassification, 4 were on ART for less than 6 months while the remaining one was on ART for more than 6 months. Stratifying the analysis by ART duration showed a significantly better specificity on those on ART for more than 6 months. Although the patients on ART for less than 6 months presented low plasma VL, they may still present a higher level of cells harboring HIV. In these patients, discrepancies could be explained by the amplification of intracellular RNA.

To perform this DBS evaluation, patients not yet on ART, on ART for less than 6 months, and on ART for more than 6 months were enrolled. These three groups of patients allowed us to obtain a large range of VL values from undetectable to very high. Other studies which have evaluated DBS VL performance also enrolled HIV negative patients [11]. This choice would de facto have cancelled the potential impact of proviral DNA and intracellular RNA amplification and could have artificially improve specificity performance. The choice of patients enrolled also allowed the study to be close to the real–life situation in terms of a continuum of VL levels. Had this evaluation been performed on patients with undetectable VL on one hand, and on high VL patients on the other hand, DBS performances would have been even better. In view of the extremely high quantitative correlation and given the VL levels when discrepancies were observed, one could imagine that 100% sensitivity and specificity could have been achieved.

From a clinical perspective, a downward misclassification (default in sensitivity) means that some patients who are in virological failure are not identified when using DBS. This is an important issue as following the WHO guidelines would mean that these patients would not be virologicaly evaluated for another 12 months or until an immunological or clinical deterioration is observed, and would be maintained on an inefficient ART regimen, thus increasing the likelihood of HIV drug resistance acquisition. An upward misclassification means that patients are incorrectly categorized as being in virological failure and that unnecessary switches to second line ART could occur. However, following current WHO guidelines [5], patients with a VL $\geq$1000 copies/mL undergo counselling to improve ART adherence and a VL measurement is performed one to three month later. Thus, the probability of having two consecutive DBS VL above 1000 copies/mL, measured inaccurately, is low. Moreover, only patients on ART for more than 6 months would be evaluated, limiting the impact of proviral DNA and intracellular RNA amplification. A small default in specificity is less worrisome, other than the additional costs of unnecessary VL testing in a small proportion of patients. However, the high specificity obtained in our study and in others [10, 13, 16] limits this risk. When both sensitivity and specificity reach acceptable levels, this motivates maximization of the sensitivity (minimization of downward misclassification).

One strength of this study lies in its setting in field conditions, in a routine laboratory in charge of the VL monitoring of HIV infected patients on ART from Northern provinces of Vietnam, and not in a research-dedicated laboratory. Duration and conditions of DBS storage also reflect field conditions. It was important to perform this evaluation in these conditions, in order to make sure that scaling-up of VL monitoring on DBS is possible. Strictly limiting this evaluation on HIV positive patients also strengthens our results and allows good extrapolation. Finally, all DBS VL results were obtained blinded from plasma VL results.

One limitation of our study is the DBS preparation at the laboratory in charge of VL monitoring and not at the outpatient clinic. This allowed maximizing the quality of the plasma sample, used as the gold standard. However, venous sampling and then spotting of whole blood with a calibrated pipette is easy to implement in laboratories in remote areas. Another limitation was the use of convenience sampling which does not allow for estimating the prevalence of failure, and does not allow for estimating the positive and negative predictive values. For the quantitative evaluation, we chose to keep all measurements [10, 14, 15] and arbitrarily attributed the value of 20 copies/mL for all undetectable VL measurements, whether on plasma or on DBS. This allowed matching observations which were both undetectable, although this also introduced over-estimated differences when plasma VL was detectable. Despite this, correlation between plasma and DBS VL measurements was very high and the bias was reduced. Attributing a similar value to all VL measurements <839 copies/mL, in plasma and from DBS, would have smoothed differences and somewhat artificially improved the correlation. It must also be noted that the goal of this study was to evaluate the ability of DBS to diagnose virological failure at the threshold of 1000 copies/mL, and not to perform a quantitative evaluation.

In conclusion, in settings where plasma VL monitoring is not feasible, the new CE marked Abbott optimized protocol, offers the opportunity to efficiently monitor HIV patients on ART with good performances at the current threshold of 1000 copies/mL. Scaling up of VL monitoring using DBS can contribute to efforts to reach the last 90 the UNAIDS targets of 90-90-90 to help end the AIDS epidemic.

Supporting information

S1 Table. Downward misclassification. Seven patients with plasma VL >1000 copies/mL but DBS VL <1000 copies/mL.
(DOCX)

S2 Table. Upward misclassification. Five patients with plasma VL <1000 copies/mL but DBS VL >1000 copies/mL.
(DOCX)

Acknowledgments

We sincerely thank all the patients who agreed to take part in this study, the health care staff from the three outpatient clinics. We also are very grateful to the provincial AIDS center of Hanoi (Vietnam), and to Dr. Frédérique Guiroy (Assistant Coordinator, French Agency for Research on AIDS and Viral Hepatitis (ANRS)) for their help in the implementation of the study. We also thank the Clinical Core from the Center for Translational Research at Institut Pasteur (Paris France) and more specifically Mrs. Cassandre Von Platen for her valuable help in the ethical and regulatory procedures. We also thank Rebecca Grant for her careful reading and editing of the manuscript.

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Discussion:

Le travail de revue de littérature et de méta-analyse a permis de réaliser une actualisation des chiffres de succès virologique pour les patients infectés à la VIH et vivant en Afrique Sub-saharienne. Il a permis de mettre en évidence des aspects méthodologiques pratiques pour l'évaluation des programmes ainsi que pour l'évaluation de l'efficacité des TARV dans ces contextes. Une très grande hétérogénéité des études et des résultats a été mise en évidence comme dans des travaux identiques (49, 85-87). Concernant la comparaison des résultats issus des études transversales, de cohortes et des essais cliniques, l'extrapolation des résultats de ces derniers dans la pratique courante sur le terrain n'est évidente pour plusieurs raisons. En effet, la stratégie globale consiste à choisir un échantillon, à l'étudier et, si possible, à extrapoler le résultat à la pratique courante en « vie réelle ». La pertinence dépend de la validité externe (ou généralisabilité) cependant celle-ci est nécessairement plus subjective que l'évaluation de la validité interne (capacité à mesurer ce qui est prévu de mesurer) (88). Les essais contrôlés randomisés, afin de comparer des stratégies entre-elles, sont davantage susceptibles d'être exempts de biais que les études d'observation (89, 90). Ils constituent « le gold standard » étant la seule façon connue d'éviter les biais de sélection et de confusion dans la recherche clinique. Toutefois, la validité externe des résultats des essais randomisés est souvent faible (91, 92). Dans le contexte de l'étude d'efficacité de TARV en Afrique, où l'efficacité du traitement est déjà prouvée par des études cliniques dans les pays à revenu élevé, l'impact du type de suivi, des soins et de l'observance semble être élevé. Ainsi, les résultats de l'essai peuvent dépendre fortement des caractéristiques des prestataires de soins, de l'environnement et des patients. Par conséquent, à moins que l'on en prenne soin dans la conception et la conduite d'un essai randomisé, les résultats peuvent ne pas être généralisables dans de nombreux domaines des soins de santé (93). En effet, les professionnels de santé qui participent peuvent être non représentatifs et/ou les patients qui participent peuvent être atypiques (94). Les participants qui s'inscrivent habituellement à des essais contrôlés randomisés ont tendance à être différents (y compris être en meilleure santé) de ceux qui choisissent de ne pas participer, conséquence des critères de sélection restreints. Grimes et al, dans une série épidémiologique, citent Feinstein qui a dit que le processus de filtrage pour l'admission dans des essais randomisés pourrait, par conséquent, aboutir à "un type de fleur de serre, qui ne peut pas fleurir ou être éliminée avec succès au-delà de sa serre spéciale". Ainsi, toutes les études de cohortes et les essais cliniques contrôlés sont exposés à des biais différents. Les principaux biais sont le biais de sélection tel que : le biais de participation, de perte de vue, de survie (« survivor bias ») ou l'effet « travailleur sain » (« "health worker effect ») (95). De plus, selon les études, le suivi des patients dans les centres participants aux études peut ne pas être représentatif de celui pratiqué dans les autres centres du pays car ces centres sont généralement plus grands, avec davantage de personnel et de ressources financières, en l'occurrence le temps de l'essai clinique. C'est pourquoi, les données issues des essais cliniques ne devraient pas être incorporées aux données d'évaluation des programmes du fait de leur faible extrapolation à la vie réelle. Indépendamment, la réalisation d'essai clinique reste importante afin d'évaluer notamment les nouvelles thérapies. En effet, les résultats peuvent être tout à fait différents de ceux obtenus dans les PHR du fait de caractéristiques différentes de population (bassin génétique par exemple), d'un environnement différent et/ou de caractéristiques virologiques différentes potentiellement exclues des essais menés par les industriels dans les PHR (par exemple : CV à l'inclusion élevée, résistances transmises, diversité génétique du virus).

Au Cameroun, par exemple, en raison de la proximité avec le bassin de naissance du VIH-1 (96), les souches virales ont une grande diversité génétique circulent (97). Cette grande diversité génétique peut entrainer des réponses virologiques variées comme par exemple la résistance dite « naturelle » des VIH-1 du groupe 0 aux INNTIs (98). Ainsi, l'accès à des outils de diagnostic et de suivi virologique est primordial dans ce contexte permettant en plus de l'évaluation de l'efficacité des TARV et de la prévention de l'accumulation de résistance virale, la détection d'une dissociation immuno-virologique par exemple (99-101).

Les travaux menés au Cameroun ont permis d'apporter des informations précieuses au niveau individuel et programmatique. Le taux de rétention mis en évidence était dramatiquement bas, reflet de la faiblesse du système de santé. Le taux de succès virologique mis en évidence était faible et le niveau de résistance virale élevé rendant compte d'une situation alarmante. D'un point de vue de clinicien, le point commun entre toutes ces données inquiétantes : manque de détection précoce de l'échec thérapeutique, acquisition et accumulation importante de résistance sous TARV, défaut d'observance, impossibilité de gérer l'impact d'interaction médicamenteuse lié à la prise concomitante connue ou non connue d'autres médicaments, très faible taux de recours aux TARV de seconde ligne, est l'absence d'accès à la mesure de la CV pour les patients sous TARV. Il est à noter que dans la plupart des centres évalués, à l'heure actuelle, seule l'utilisation des DBS permettrait l'accès à la CV pour les patients sous TARV. En effet, les laboratoires sont extrêmement rudimentaires, souvent dépourvus d'automates de « soins courants » comme des automates d'hématologie ou des spectrophotomètres de biochimie. La plupart de ces centres ont été dotés à un moment d'automates, non fonctionnel aujourd'hui, notamment en raison d'une absence de maintenance, d'impossibilité de réparation, de défaut d'approvisionnement de réactifs, d'instabilité de l'électricité endommageant les machines et rendant leur utilisation incertaine et enfin parfois d'une absence de modèle économique permettant la pérennité de leur utilisation. Nos travaux mettent en évidence le besoin urgent de renforcement global du système de santé allant de l'informatisation des registres (permettant un meilleur suivi des files actives et une meilleure détection des perdus de vue), à l'accès à la CV en passant par l'implication communautaire absolument nécessaire afin d'assurer un lien efficace et adapté entre les patients et le système de santé.

Cette situation met en évidence le contraste criant entre les PHR et les PRL particulièrement l'Afrique qui héberge plus de la moitié des patients infectés par le VIH (2). Les PHR suivent des recommandations de prise en charge pour les patients tant au niveau thérapeutique, du suivi biologique que de la prise en charge psycho-sociale ayant d'une part permis à l'espérance de vie de ces derniers de devenir quasiment identique à la population générale et d'autre part d'observer une baisse constante de l'incidence et du taux de mortalité (2, 102). En France, le taux de succès virologique des patients sous TARV est estimé à 90% (103, 104) et le taux de résistance transmise a diminué (105). Comme présenté précédemment, les pays d'Afrique et plus particulièrement d'Afrique Centrale et de l'Ouest ne montrent pas le même bilan. Le taux de contrôle virologique y est bien inférieur comme montré dans notre méta analyse, et les taux de résistances transmises et acquises sont en augmentation au Cameroun comme ailleurs dans la région (83, 106, 107). Il est à noter que la stigmatisation des patients infectés joue un rôle important notamment sur l'adhérence au TARV et pourrait expliquer cette faiblesse des taux de succès virologique en Afrique (2, 108, 109). Dans l'étude que nous avons menée au Cameroun, seulement 5.4% des patients avaient un entourage connaissant leur statut virologique. Globalement, cette situation questionne l'efficacité de l'approche de Santé Publique adoptée par l'OMS et rappelée en introduction. Cette approche, inspirée de

l'expérience du traitement antituberculeux, prend en compte les contraintes et faiblesses des systèmes de santé des PRL, y compris celles concernant le niveau de formation des acteurs de santé, le nombre important de patients, la disponibilité limitée des médicaments, l'absence de plateforme biologique et s'appuie sur l'expérience de programmes pilotes (110-113). Les caractéristiques de cette approche globale sont la standardisation des traitements antirétroviraux de première et deuxième ligne, la simplification des arbres décisionnels et du suivi, la standardisation du monitorage biologique et la décentralisation des structures de prise en charge. Elle a ainsi permis le déploiement à grande échelle des TARV. Malheureusement, cette simplification est fondée principalement sur les contraintes économiques et non médicales ou virologiques comme le montre par exemple le choix du seuil définissant l'échec thérapeutique et le changement de ligne de traitement, la fréquence du suivi virologique après qu'il n'ait pas été recommandé initialement ou l'utilisation d'un TARV de 1ère ligne utilisant des molécules à barrière génétique faible (114, 115), dans un contexte de grande faiblesse persistante d'accès au suivi biologique et virologique notamment. Cependant, sans attendre une évolution des moyens financiers, humains et techniques des PRL, les différences entre PHR et PRL pourraient être atténuées par une adaptation des mesures et outils mis à disposition pour faire face aux contraintes locales.

L'objectif des travaux menés au Vietnam est de montrer la faisabilité de la mise en place d'un système de collecte de prélèvement sur DBS pour mesurer la CV VIH. Dans ce cadre, les premiers travaux menés ont porté sur l'évaluation des résultats de CV obtenus sur DBS comparativement aux résultats obtenus sur plasmas. Ces travaux ont permis de s'assurer de la qualité des résultats rendus aux patients. De plus, nous avons mis en place des conditions permettant une mise à l'échelle rapide en minimisant le temps de travail manuel du laboratoire : spot de DBS pré-découpé permettant de s'affranchir du temps de punch (évitant également ainsi tout risque de contamination des prélèvements par le matériel utilisé) ; utilisation d'automates pour l'extraction, l'isolement et l'amplification des acides nucléiques. Ainsi, le laboratoire était en capacité de techniquer une à deux plaques par demi-journée (90 prélèvements de patients par plaque pour Abbott® et 40 pour Roche®). De même, nous avons choisi d'évaluer des kits commerciaux (élution et amplification venant du même industriel) afin d'être dans les conditions les plus proches des laboratoires de routine et non de recherche : fonctionnement, équipement et approvisionnement en réactifs. En effet, plusieurs publications évaluent les performances des résultats de CV obtenus sur DBS en combinant plusieurs techniques venant d'industriels différents ou des techniques « maison » (74, 116, 117).

Les résultats obtenus en utilisant les techniques d'élution et d'amplification recommandées par Abbott® sont en accord tant, en termes de Sensibilité que de Spécificité, avec les résultats générés par d'autres équipes (4, 21, 75-77, 118-122). La technique Abbott® est donc une technique reconnue comme robuste dans le cadre de la mesure de la CV sur DBS et a obtenu le marquage CE dans cette indication.

Concernant le nouveau protocole d'élution développé par Roche® (FVE) (permettant de minimiser l'amplification de l'ADN proviral et par conséquent d'améliorer la spécificité des résultats), les très bonnes performances, en termes de Spécificité retrouvé dans notre étude, sont similaires à celles retrouvées par d'autres équipes (123-125). En termes de Sensibilité, Guichet et col, ont trouvé des résultats similaires aux nôtres avec une sensibilité de 60% (IC95% (49%-71%)). Cependant d'autres équipes ont trouvé des résultats bien meilleurs que les nôtres. Pollack et col également au Vietnam ont trouvé une Sensibilité de 95.1% (IC95% :87.8%-98.6%) avec un facteur correcteur de +0.3log copies/mL(124). Makadzange et

col ont trouvé une Sensibilité de 92.7% (IC95% :88.6%-96.6%)(125). Wu et col, dans le cadre d'une publication de l'industriel, ont trouvé une Sensibilité de 90% (123). Pannus et col ont trouvé une Sensibilité de 80.8% (IC95% : 73.3–86.7)(126).
La sous-estimation des résultats de CV obtenus sur DBS en utilisant la technique Roche® (COBAS®AmpliPrep/COBAS® TaqMan® HIV-1 Test v2.0) est connue et retrouvée dans plusieurs études utilisant (125)(116) ou non (77, 127) le nouveau protocole d'élution FVE. Cette sous-estimation a, d'ailleurs, conduit Pollack et col à proposer deux facteurs correcteurs de +0.3 et + 0.7log copies/mL avec de meilleures performances en utilisant le facteur correcteur le plus élevé.
Il est important de noter que les conditions dans lesquelles sont réalisées les études et sont techniqués les prélèvements varient d'une étude à l'autre. Ceci peut avoir un impact sur les performances des techniques évaluées si celles-ci ne sont pas suffisant robuste. En effet, dans nos études MOVIDA1 et 1bis et ce quelque soit la technique utilisée, nous avons choisi de reproduire au maximum les conditions de vie réelle. Ainsi, après le séchage des DBS, nous avons conservé les cartes à température ambiante locale pendant 15 jours mimant ainsi le délai maximum entre leur confection et leur transport au laboratoire centralisé lorsque les prélèvements arrivent des zones décentralisées. Par la suite, les cartes de DBS ont été techniquées ou congelées à -20°C jusqu'à obtention de suffisamment de prélèvements pour techniquer une plaque complète. Les laboratoires n'étant pas tous dotés de congélateurs à -80°C ou n'ayant pas tous la place disponible dans ce type de congélateur dans des conditions d'extension massive de la technique à l'ensemble d'un territoire. Dans l'étude de Pollack et col, les DBS étaient immédiatement après séchage soit techniqués soit mis dans un congélateur à -70°C. Ces conditions peuvent expliquer les différences de résultats en termes de Sensibilité et de dégradation de l'ARN viral. Cependant, les conditions que nous avons mis en place dans nos études semblent être plus proches des conditions de vie réelle et donc les résultats obtenus plus extrapolables que les conditions utilisées par l'équipe de Pollack et col plus orientées sur l'optimisation des résultats obtenus par la technique utilisée. De même, Pannus et col ont congelé les cartes de DBS à -20°C immédiatement après séchage. Makadzange et col, eux, ne décrivent pas les conditions dans lesquels les DBS ont été conservés avant d'être techniqués mais précisent dans leur discussion que les résultats ont été obtenus dans des conditions de laboratoire et que la Sensibilité pourrait ne pas être la même dans des conditions de terrain.

Lorsque l'on regarde la situation actuelle de l'accès à la CV dans les PRL, une question se pose : pourquoi cet accès est-il aussi limité près de 20 ans après l'introduction des TARV ? Un certain nombre de raisons sont connues (128). Deux paramètres peuvent être mis en avant, d'une part le facteur temps et d'autre part la nécessité absolue d'une interaction forte entre clinique et laboratoire. Les expériences antérieures avec l'introduction de nouveaux tests de diagnostic dans des environnements à faibles ressources suggèrent que les difficultés de mise en œuvre pourraient ralentir la mise à l'échelle. Cependant, ces difficultés de mise en œuvre peuvent également provenir d'une offre inadaptée à une partie de la population et des infrastructures dont elle dispose. Par exemple, il a fallu près de 10 ans pour obtenir une couverture de seulement 60% de l'accès au test CD4 et ce malgré différents types d'offre incluant les machines POC subventionnées. Deuxièmement, les tests de CV doivent être utilisés efficacement par les cliniciens et les patients pour améliorer la prise en charge clinique et l'observance afin d'atteindre l'objectif de suppression virale à 90%. Des expériences pratiques récentes ont montré que sans un appui et un encadrement des cliniciens, la mise à disposition seule de la CV ne suffit pas pour obtenir sa prescription et/ou son incorporation

dans la prise en charge thérapeutique. Par exemple, dans un autre cadre, une étude sur la mise à l'échelle de tests de diagnostic moléculaire de la tuberculose en Afrique du Sud a montré un impact minimal sur la mortalité des patients, probablement en raison d'un accès inadéquat des patients au test et d'une mauvaise utilisation des résultats par les cliniciens et les patients pour améliorer leur prise en charge (129). Ainsi, à la différence de l'accès au TARV via la baisse des prix notamment, l'accès aux examens de laboratoire n'a pas suivi cette voie. Une partie de cette situation est liée aux industriels, imperméables à la pression de la communauté internationale sur la baisse des prix des machines et des réactifs de laboratoire et sur l'adaptation nécessaire aux contraintes locales. Ces derniers réussissent à poursuivre une politique de profit fondée sur une propriété intellectuelle foisonnante et multiforme qui porte à la fois sur les équipements, les logiciels et les réactifs et s'appuyant sur des chaînes d'automates incluant robots d'extraction d'acides nucléiques, appareils de PCR en temps réel et ordinateurs équipés des logiciels informatiques adéquats. Tout étant lié, ils forment également des ensembles de "black boxes" utilisables uniquement avec les réactifs et matériels fournis par l'industriel (130-132). Des plans de contournement auraient pu s'organiser mais la faiblesse de la collaboration entre équipe de recherche de laboratoire et les cliniciens associée à la faible diffusion à l'échelle national des techniques réalisées dans de rares laboratoires centralisés, ne l'ont pas permis, assurément à large échelle. Cependant, certains projets comme le projet OPP-ERA (http://www.opp-era.org/) financé par UNITAID a pour objectif de s'attaquer à cet aspect de la limitation d'accès en favorisant le modèle des plateformes polyvalentes ouvertes.

Dans un article publié dans le « Morbidity and Mortality Weekly Report » sur la mise à l'échelle du suivi virologique dans 7 pays d'Afrique Subsaharienne (Côte d'Ivoire, Kenya, Malawi, Namibie, Afrique du Sud, Tanzanie, and Ouganda), les obstacles les plus fréquemment évoqués étaient la difficulté de transport des prélèvements et l'insuffisance de ressources humaines formées et dédiées à l'analyse de la CV. Pour 3 d'entre eux, l'utilisation des DBS comme support de prélèvement était déjà identifié comme facteurs de succès de la mise à l'échelle de l'accès à la CV ainsi que la mise en place d'un réseau de ramassage des échantillons (133).

Par conséquent, alors que la mesure de la CV sur DBS est connue depuis plus de 10 ans, quelles sont les raisons de sa non-utilisation ?

Les raisons avancées sont le manque de Spécificité, le manque de Sensibilité, l'absence de protocole validé par les fabricants et la non immédiateté du résultat pour le clinicien et son patient (77).

Le développement des tests POC est présenté comme la solution permettant l'universalité de l'accès à la mesure de la CV pour les patients sous TARV notamment ceux issus des zones décentralisées (60, 128, 134). L'immédiateté des résultats obtenus via ce type de tests ne fait aucun doute. Cependant d'autres obstacles semblent être présents comme la non simplicité réelle de leur utilisation par des personnels non formés, la nécessité du recours au plasma pour la plupart, la maintenance nécessaire de l'ensemble des machines, l'approvisionnement en réactif et le coût réel (non subventionné des machines) (64). La question même de la performance de ces machines peut être posée. En effet, actuellement une machine a obtenu une pré-qualification par l'OMS : Xpert® HIV-1 Viral Load avec GeneXpert® (Cepheid, Sunnyvale, US). Dans le dossier de préqualification, la Sensibilité et la Spécificité pour la détection de l'échec virologique au seuil de 1000 cp/mL était respectivement de 94.14% (IC95%: 90.37-96.76) et 98.50% (IC95%: 95.68-99.69) (63, 135). D'autres machines sont actuellement en cours d'évaluation. Une étude reprenant les performances du test POC

développé conjointement par MSF et l'Université de Cambridge nommé SAMBA I et SAMBA II retrouvait les caractéristiques suivantes : seuil limite de détection légèrement au-dessus de 1000 cp/mL et une concordance par rapport aux résultats obtenus sur plasma au seuil de 1000 cp/mL oscillant de 96.9% (IC95% : 93.4–98.9%) à 99.2% (IC95% : 95.7–99.9%) (59).
Lorsque du sang total est utilisé pour mesurer la CV des patients via des tests POC afin de faciliter leur utilisation et s'affranchir des problématiques de centrifugation, les performances de ces machines sont fortement affectées. Par exemple, Jani et col rapportent des résultats de Sensibilité et de Spécificité de respectivement 96.83% et 47.80% au seuil de 1000 cp/mL par comparaison aux résultats obtenus de CV plasmatique (Roche Cobas Ampliprep/Cobas TaqMan v2) en utilisant du sang capillaire. En utilisant du sang total veineux, la Sensibilité était de 98.39% et la Spécificité de 37.93% toujours au seuil de 1000 cp/mL (61). Le test POC Liat HIV Quant (IQuum, Inc. racheté par Roche Molecular Systems, Inc., USA), au seuil de 1000 cp/mL, avait une Sensibilité de 100% (IC95% : 95.3%-100%) et une Spécificité de 88.2% (IC95% : 63.6%-98.5%) à partir du plasma et de 100% (IC95% : 95.3%-100%) et 41.2% (IC95% : 18.4%-67.1%), respectivement à partir du sang total (62). L'absence de système de contrôle qualité pose également question lors de la mise à l'échelle du déploiement de ce type de machine. La question de leur performance persistent dans des régions comme l'Afrique de l'Ouest et l'Afrique Centrale hébergeant une grande diversité de souche de VIH (136) notamment vis-à-vis des souches d'HIV-2 ou des sous-types non B d'HIV-1. En effet, GeneXpert® a obtenu une pré qualification la mesure des CV des souches de VIH-1 du groupe M (sous-types A, B, C, D, F, G, H, J, K, CRF01_AE, CRF02_AG, et CRF03_AB) mais pas pour HIV-2 (63). L'évaluation des autres machines ne rapportent pas ou très peu (sur des effectifs très restreints) de données (59, 61, 62). Enfin, le coût réel des machines, des tests et de la maintenance hors subvention n'est pas encore réellement connu, ce qui pose question sur la pérennité des systèmes mis en place en cas de désengagement des bailleurs internationaux.
C'est pourquoi, l'ouverture à d'autres approches, complémentaires, semble possible.
Concernant la mesure de la CV sur DBS, certaines techniques commerciales actuelles ont les performances recommandées par l'OMS, c'est-à-dire plus de 85% de Sensibilité et de Spécificité (4, 77). Cependant, des voies d'amélioration sont possibles.
Le manque de Spécificité, c'est-à-dire la capacité à détecter le succès virologique lorsqu'il est présent est en lien avec l'amplification de l'ADN proviral lorsque le sang total est utilisé (72, 74, 117, 137). Dans ce cadre, l'utilisation de DNase permet d'améliorer les performances. Cependant, le temps de contact et le caractère couteux et chronophage de cet ajout apparaissent comme des obstacles opérationnels (116, 138). L'utilisation de plasmas à la place du sang total c'est-à-dire de Dried Plasmas Spot (DPS) peut apparaitre comme une solution puisque dépourvu d'ADN mais pose des problèmes de différents ordres tels que la nécessité de centrifugation sur site et la moindre conservation à température ambiante de l'ARN viral sur DPS (72, 74, 117, 121, 139). D'autres voies d'amélioration sont possibles telles que l'utilisation d'une amplification isotherme comme celle développée par Biomérieux, NucliSENS®, (NASBA « *Nucleic Acid Sequence-Based Amplification* ») permettant de ne pas séparer les doubles brins d'ADN donc de ne pas les amplifier (74, 140). Il s'agit d'ailleurs de la première technique à être préqualifiée par l'OMS pour la mesure de la CV sur DBS avant celle d'Abbott® (141, 142). Cependant, cette technique a comme obstacle son coût et sa relative faible diffusion sur le marché actuel. L'utilisation de procédé d'élution différent pourrait permettre également de ne pas amplifier l'ADN proviral. C'est dans cet objectif que Roche a développé le protocole FVE (Free Virus Elution) à base de PBS (phosphate buffered saline).

Le manque de Sensibilité, c'est-à-dire la capacité à détecter l'échec virologique lorsqu'il est présent est en lien avec la limite inférieure de détection actuelle du DBS entre 550 et 1000 cp/mL alors qu'elle est entre 20 et 40 cp/mL lorsque le plasma est utilisé. Cette problématique semble en partie en lien notamment avec le faible volume de sang total déposé : entre 20 et 100 µL de sang total pour les DBS et entre 100 µL et 1 mL pour le plasma. Une autre cause possible pourrait être les procédés d'élution et d'extraction. Certains procédés d'extraction sont reconnus pour permettre d'amplifier préférentiellement l'ARN que l'ADN. Cependant, dans ces dernières recommandations, l'OMS poursuit sa recommandation de définition de l'échec virologique au seuil de 1000cp/mL (4). D'un point de vue virologique, l'impact de la faible réplication notamment en terme d'accumulation potentielle de résistance n'est plus à démontrer (143-145). Cependant, d'un point de vue de Santé publique, l'accès concret et constant à la CV pour les patients sous TARV, dans un contexte d'extension massive de la couverture ARV et avec des recommandations de mesures régulières de cette dernière, semble bien plus prioritaire que l'abaissement du seuil définissant l'échec virologique. Ce d'autant que dans la méta-analyse que nous avons réalisée, nous montrons une différence significative des proportions d'échec virologique si ce dernier est défini au seuil de 1000 ou 5000 cp/mL mais aucune différence significative entre une définition à 1000 cp/mL et 400 cp/mL (146). Une définition du seuil telle qu'elle a commencé à être rédigée en mentionnant la possibilité de réaliser des prélèvements sur DBS, lorsque le plasma ne peut être utilisé semble être une voie adaptée. Lorsque le plasma est possible, une définition du seuil non fondée sur les capacités de détection des machines semble incohérente. Lorsque le plasma n'est pas possible, une définition du seuil excluant la possibilité d'utiliser le DBS semble l'être tout autant. L'accès et la réalisation du suivi virologique doit être la priorité. Ainsi, un seuil définissant l'échec, reprenant les standards utilisés dans les PHR et permettant un maintien de celui-ci à 1000 copies/mL lorsque le DBS est utilisé, parait être le meilleur des compromis.

L'utilisation des DBS comme support de prélèvement afin de donner accès au suivi virologique aux patients notamment issus des zones décentralisées apparait faisable, fiable, remplissant les critères de performances requis et disponible immédiatement. De plus, les industriels semblent de plus en plus s'intéresser à l'utilisation de ce support de prélèvement, avec la production de manuel officiel d'utilisation certifié CE et/ou FDA, laissant ainsi présager une amélioration future des performances.

De plus, outre la mesure des résistances virales déjà validées et recommandées par l'OMS, l'utilisation des DBS comme support de prélèvement permet un large panel d'analyses biologiques dans le cadre du VIH comme les mesures toxicologiques (147, 148), dans cadre d'autres maladies infectieuses comme l'hépatite B et C (sérologie et charge virale) (149-153) ou les examens sérologiques dans le cadre du diagnostic ou de la surveillance (154-156). Les DBS peuvent également être utilisés comme supports de prélèvement afin d'être un outil diagnostic de maladies non infectieuses comme le diabète ou les hémoglobinopathies (157-161).

Perspectives

A la suite des projets présentés dans le cadre de cette thèse, plusieurs projets sont en cours ou en préparation. Les axes développés sont 1) la mise en place d'une cohorte de patients sous TARV issus de zones décentralisées suivis virologiquement grâce aux prélèvements sur DBS, projet appelé MOVIDA 2 Vietnam 2) l'amélioration des performances des résultats de CV obtenus sur DBS via l'amélioration des papiers buvards utilisés, projet

appelé MOVIDA paper 3) la mesure de la CV Hépatite C sur papier buvard en condition de terrain, projet appelé MOVIDA hep.
Le projet MOVIDA 2 Vietnam (ClinicalTrials.gov Identifier: NCT03249493) a pour objectif 1) de donner accès à la CV VIH aux patients sous TARV issus de zones décentralisées 2) apporter des données de faisabilité et d'opérationnalité de la mise en place d'un système de collecte de DBS 3) déterminer les facteurs de risque d'échec virologique et d'acquisition de résistance virale. Ce projet est financé par le Fond Mondial et a débuté en juillet 2017. La période d'inclusion est terminée et 576 patients initiant un TARV et issus de 47 sites de 6 provinces du Nord Vietnam ont été inclus. Conformément à ce qui était attendu, la moitié (47.8%) sont des utilisateurs de drogues injectables (UDI). Une partie importante des patients suivis est infectée par l'hépatite C (41.8%) avec une proportion importante chez les UDI (77.7%). Le suivi de ces patients durera 24 mois et le suivi virologique se déroulera selon les recommandations de l'OMS c'est-à-dire une CV à 6, 12 et 24 mois de TARV. Pour se faire, les prélèvements de sang total sont réalisés sur DBS et envoyés chaque semaine via le réseau postal au laboratoire de virologie de NIHE. En cas de CV>1000 cp/mL, une CV de confirmation est réalisée dans un délai de 1 à 3 mois après renforcement de l'observance. Un génotypage de résistance sera réalisé en cas de CV confirmée > 1000 cp/mL. En cas de mise en évidence de résistance virale, un génotypage de résistance sera réalisé sur le prélèvement effectué à baseline afin de distinguer les résistances acquises des résistances transmises. Pour le moment, le délai médian d'acheminement des DBS des sites vers le laboratoire centralisé est de 7 jours (IIQ 4-15). Aucun échec de la technique n'a encore été constaté. Une étude de coût-efficacité sera réalisée dans le cadre de ce projet.
Cette étude de cohorte est pour nous une étape essentielle pour le passage à l'échelle de ce système.

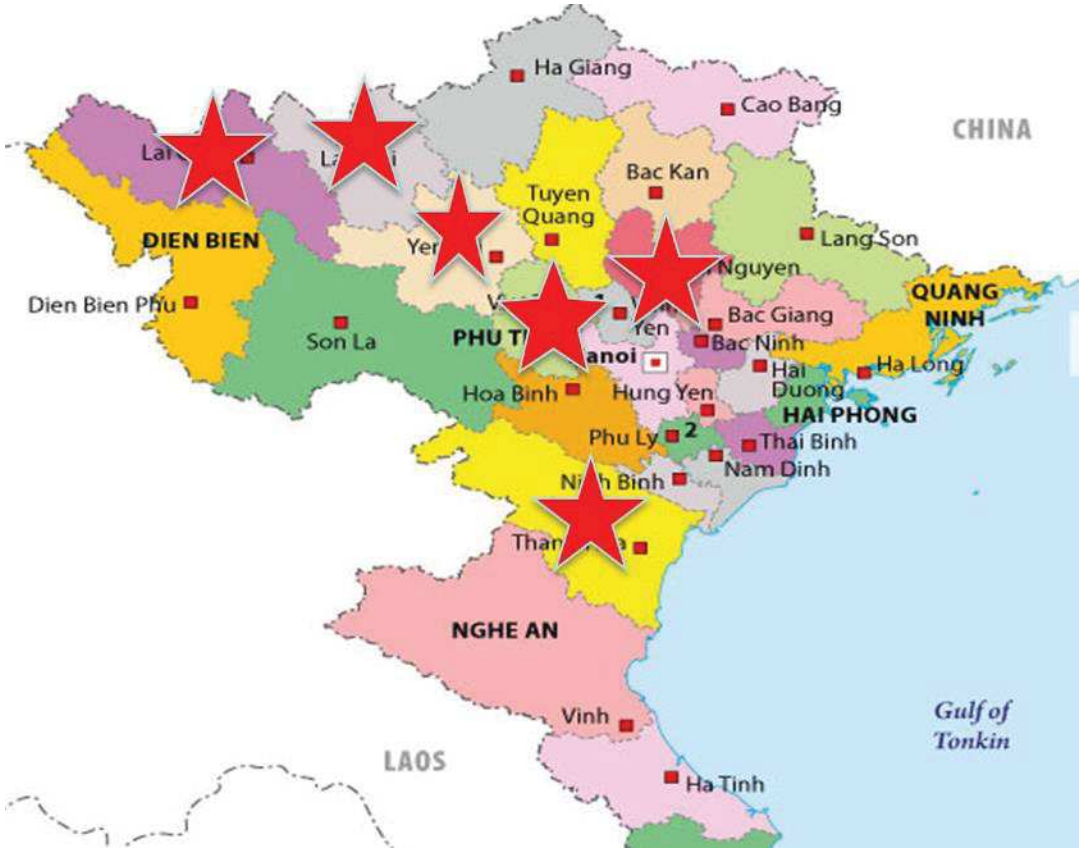

Figure 5 : Provinces du Nord Vietnam impliquées dans le projet MOVIDA 2 Vietnam

Dans le cadre de l'amélioration des performances des résultats de CV obtenus sur DBS, nous avons désiré axer une partie de nos recherches sur l'amélioration des caractéristiques du papier buvard. Pour cela, nous avons obtenu un partenariat avec un grand producteur de papier buvard. Ce partenariat nous permettra de réaliser le projet MOVIDA paper qui a pour objectif d'évaluer les performances de différents papiers buvard en utilisant deux techniques commerciales de mesures de la CV VIH. Notre cahier des charges pour le choix des papiers buvards est l'utilisation de ces derniers en routine c'est-à-dire avec des automates et en diminuant le temps manuel des techniciens de laboratoires pour réaliser les mesures de CV (pré-découpage des spots), l'amélioration de la sensibilité et l'amélioration de la spécificité.
Après une phase préliminaire permettant de sélectionner 3 papiers buvards sur 5 sélectionnés, le projet MOVIDA paper devrait se dérouler au Vietnam au cours de l'année 2019.

Dans le cadre de l'élargissement du panel d'analyse possible en utilisant les DBS et du fait d'une proportion importante d'UDI au Vietnam donc de co-infection HIV-HCV, nous allons mettre en place dans un premier temps le projet MOVIDA hep au Vietnam. Ce projet a reçu un accord de principe de financement par un industriel et a obtenu les autorisations éthiques et administratives pour se mettre en place. Il aura pour objectif 1) d'évaluer les performances de la mesure de la CV HCV sur DBS en comparaison avec les résultats obtenus sur plasma 2) former et s'assurer de la qualité des résultats rendus par le laboratoire local en condition de vie réelle. Dans le projet MOVIDA hep, du fait d'une proportion importante d'UDI, ayant fréquemment un accès veineux compliqué pour les équipes de soins, nous allons évaluer les résultats obtenus sur DBS réalisés à partir de sang veineux et de sang capillaires.

Conclusion

Les patients issus des zones décentralisées sont considérés comme des populations vulnérables en raison de leurs difficultés d'accès aux soins. Cette population issue des zones décentralisées représente 45% de la population globale, 59% en Afrique et 51% en Asie (65% au Vietnam) (162, 163). Les difficultés d'accès aux soins sont un des paramètres important de l'exode rurale avec l'accès à l'éducation et le développement économique (164). La décentralisation des soins notamment en matière de prise en charge des patients infectés par le VIH est reconnue comme efficace par rapport à un système centralisé (165-168). Les objectifs d'universalité d'accès aux soins pour tous les citoyens, de soins efficaces pour de meilleurs résultats de santé, d'utilisation efficace des ressources, de services de haute qualité et réactivité aux préoccupations des patients, ne doivent pas être abandonné et des solutions adaptées doivent être mises en place.
Le DBS est un support de prélèvement permettant d'acheminer ce dernier de façon simple et permettant d'amplifier l'ARN viral en s'affranchissant de la chaine de froid. Différentes techniques commerciales existent et se développent en améliorant leurs performances. Certaines ont dès aujourd'hui les performances requises pour permettre l'exploitation de ces prélèvements dans le cadre du suivi de routine des patients infectés. Des voies d'amélioration des performances sont possibles et l'industrie prend de plus en plus de place dans ce domaine. La centralisation des résultats permet non seulement une meilleure rentabilité des machines existantes mais également la mise en place d'un système d'assurance qualité indispensable et la facilitation de la mise en place de système de surveillance à l'échelle nationale.
Ce support de prélèvement permet un large panel d'analyses. Dans le cadre du VIH, la mise à disposition du génotypage de résistance à l'échelle individuelle et dans le cadre du suivi

semble être la prochaine étape de l'amélioration de la prise en charge des patients dans les PRL.
Il pourrait être mis en place de façon complémentaire aux autres approches comme les POC en plus bien sûr de la CV plasmatique « classique ». Cette répartition de l'offre de CV devrait se faire en fonction du niveau des structures dans le système de santé, de leurs infrastructures, de leur modèle économique et de la file active de patient VIH dont elles disposent.
Ainsi, pour les patients issus des zones décentralisées, la mise en place d'un réseau de ramassage de DBS permettrait d'apporter une amélioration importante et complémentaire des autres approches afin d'atteindre l'objectif de l'accès universel à la mesure de la CV enjeu majeur de Santé Publique notamment en raison de l'extension majeure du nombre et de l'espérance de vie des patients sous TARV.

« Faire progresser la science au bénéfice des populations quelles qu'elles soient et ou qu'elles soient, c'est dans cet esprit que s'inscrit ce joli travail de Fabien au Cameroun et au Vietnam avec rigueur, persévérance, dynamisme et humanisme dans le pur respect des grandes valeurs pasteuriennes. »

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