



Improvement of tuberculosis diagnosis in hiv-infected children in low-income countries

Olivier Marcy

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Par Olivier MARCY

**AMELIORATION DU DIAGNOSTIC DE LA TUBERCULOSE
CHEZ LES ENFANTS INFECTES PAR LE VIH DANS LES
PAYS A RESSOURCES LIMITEES**

**IMPROVEMENT OF TUBERCULOSIS DIAGNOSIS IN HIV-
INFECTED CHILDREN IN LOW-INCOME COUNTRIES**

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(co-directeur : Christophe DELACOURT)

Soutenue le 14 décembre 2017

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« Caminante, no hay camino, se hace camino al andar. »

Antonio Machado

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Abstract

Tuberculosis remains a major cause of death in HIV-infected children, accounting for more than 1/3 of mortality in this population. There are currently no estimates of HIV prevalence in the one million childhood tuberculosis cases every year. However, it is estimated that HIV-related deaths account for up to 1/5 of all tuberculosis deaths in children. Due to its paucibacillary nature and difficulties in collecting respiratory specimen, tuberculosis diagnosis is challenging in children, and is a major barrier hampering access to treatment. It is estimated that more than 96% of tuberculosis death occur in children not receiving treatment. HIV-infected children present greater diagnostic challenges in a context of higher mortality. The main objective of this thesis was to contribute to the improvement of tuberculosis diagnosis in HIV-infected children by the development of a diagnostic algorithm. This thesis is based on the ANRS 12229 PAANTHER 01 study which enrolled 438 HIV-infected children aged 0 to 13 years presenting with a suspicion of tuberculosis in 8 hospitals from Burkina Faso, Cambodia, Cameroon, and Vietnam, from April 2011 to Mai 2014.

Mortality was high [50 deaths (19.6%)] in the 266 art-naïve children from this study, despite access to antiretroviral and tuberculosis treatment. It was significantly higher in those with bacteriological confirmation by Xpert MTB/RIF (Xpert) or smear microscopy. Introduction of tuberculosis treatment led to decreased mortality in both confirmed and unconfirmed tuberculosis, and to delayed ART introduction, which is a problem, as ART was associated with a more than 10-fold reduction in mortality when started during the first month of follow-up. The assessment of the diagnostic performance of Xpert on alternative specimens including nasopharyngeal aspirates (NPA), stool samples, and string tests showed that a combination of NPA and stool samples had a high feasibility (100%) and sensitivity (75.9%) and a yield similar to standard specimen collection methods consisting in sputum samples or gastric aspirates. We developed a tuberculosis diagnostic algorithm for HIV-infected children. The model identified 11 predictors including some symptoms and signs, history of contact, chest radiograph (CXR) and abdominal ultrasonography findings, and QuantiFERON TB Gold In-Tube (QFT) results. Its area under the receiver operating characteristic curves was 0.839, and 0.866 when Xpert was included. The score developed from the model had a good diagnostic performance overall. CXR interpretation showed poor to fair inter-reader agreement for the detection of lesions consistent with tuberculosis in 403 children with all reviews available, and limited diagnostic accuracy (sensitivity 71.4%; specificity 50.0%) for the diagnosis of tuberculosis in 51 culture confirmed cases and 151 controls alive without treatment at 6 months. At last, we present data on the use of the recommended tuberculosis reference standards for diagnostic studies in HIV-infected children, describing changes in classification occurring from a recent update and highlighting limitations of these reference standards.

Overall, this work contributes to highlight the need for an accurate and timely tuberculosis diagnosis in HIV-infected children to reduce mortality. It proposes two major ways to improve diagnosis by an alternative approach to microbiological confirmation and by an algorithm for prompt treatment decision in children with a high risk of mortality. It also highlights practical and methodological challenges related to the use of CXR and diagnostic reference standards in this population. The newly funded TB Speed project will enable external validation of the algorithm in HIV-infected children. By decentralizing Xpert performed on NPA and stool in district health systems in low-income countries, it will further contribute to the overall goal of improving tuberculosis diagnosis and reducing mortality in children.

Key words: tuberculosis, diagnosis, HIV, children, mortality, low income countries, mortality

Résumé

La tuberculose est une cause majeure de mortalité chez les enfants infectés par le VIH, représentant plus d'un tiers des décès chez ceux-ci. Il n'existe actuellement pas d'estimation précise de la prévalence du VIH dans le million de cas de tuberculose pédiatrique annuel. Cependant, les enfants co-infectés par le VIH pourraient représenter jusqu'à 1/5 de tous les décès dus à la tuberculose pédiatrique. Le diagnostic de tuberculose est difficile chez les enfants du fait de sa nature paucibacillaire et des difficultés de recueil d'échantillons respiratoires. Il constitue un obstacle majeur à l'accès au traitement. Plus de 96% des décès pourraient survenir chez des enfants non traités. Les enfants infectés par le VIH présentent des difficultés diagnostiques accrues et une mortalité plus élevée. Notre objectif principal était de contribuer à l'amélioration du diagnostic de la tuberculose chez les enfants infectés par le VIH par le développement d'un algorithme diagnostique. Ce travail est basé sur l'étude ANRS 12229 PAANTHER 01 qui a inclus 438 enfants séropositifs âgés de 0 à 13 ans suspects de tuberculose dans 8 hôpitaux du Burkina Faso, du Cambodge, du Cameroun et du Vietnam d'avril 2011 à mai 2014.

La mortalité était élevée (19,6%) chez les enfants naïfs de traitement antirétroviral (TARV), malgré l'accès au traitement. Elle était plus élevée les cas confirmés par l'Xpert MTB/RIF (Xpert) ou la bacilloscopie. Le traitement antituberculeux était associé à une diminution de la mortalité chez les tuberculoses confirmées et non confirmées et à un retard problématique au début du TARV, celui-ci étant associé à une réduction massive de la mortalité quand débuté durant le premier mois de suivi. L'évaluation de la performance diagnostique de l'Xpert sur des échantillons bactériologiques alternatifs, y compris des aspirations naso-pharyngées (ANP), des échantillons de selles et le string test a montré que la combinaison d'un ANP et des selles avait une faisabilité (100%) et une sensibilité (75,9%) élevées par rapport aux méthodes standards (crachats ou aspirations gastriques). L'algorithme diagnostique développé a inclus 11 prédicteurs dont des symptômes et des signes, le contage, les résultats de radiographie pulmonaire (RP) et d'échographie abdominale, ainsi que les résultats du Quantiferon Gold In-Tube (QFT). L'aire sous la courbe de ROC était de 0,866 lorsque l'Xpert était inclus. Le score développé à partir du modèle avait une bonne performance diagnostique globale. L'interprétation de la RP avait un accord inter-relecteurs médiocre à passable pour la détection de lésions compatibles avec la tuberculose chez les 403 enfants avec toutes les revues disponibles, et une précision diagnostique limitée (sensibilité 71,4%, spécificité 50,0%) pour le diagnostic de la tuberculose chez 51 cas et 151 contrôles vivants sans traitement à 6 mois. Enfin, nous présentons des données sur l'utilisation du standard de référence recommandé pour les études diagnostiques sur la tuberculose chez les enfants infectés par le VIH, décrivant les changements dans la classification résultant d'une mise à jour récente et soulignant les limites de ces standards.

Ce travail souligne la nécessité d'un diagnostic rapide de la tuberculose chez les enfants infectés par le VIH afin de réduire la mortalité. Il propose d'améliorer le diagnostic par une approche alternative à la confirmation microbiologique et le développement d'un algorithme rapide de décision de traitement chez les enfants à haut risque de mortalité. Il met en évidence les défis pratiques et méthodologiques liés à l'utilisation de la RP et du standard de référence dans cette population. Le projet TB Speed permettra la validation externe de l'algorithme chez les enfants infectés par le VIH et contribuera à l'amélioration du diagnostic de la tuberculose et à la réduction de la mortalité chez les enfants en décentralisant la confirmation microbiologique et le diagnostic dans des pays à faible revenu.

Mots-clefs : tuberculose, diagnostic, VIH, enfants, pays à ressources limitées, mortalité

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Résumé substantiel

1. Introduction/ 2. Co-infection tuberculose VIH chez l'enfant

La tuberculose est une maladie bactérienne due à des mycobactéries du complexe *Mycobacterium tuberculosis* (MTB). Maladie à transmission interhumaine par voie respiratoire, elle est la première cause infectieuse de mortalité dans le monde avec 1,8 million de décès, dont 400.000 chez des personnes infectées par le VIH, alors que les décès liés au VIH s'élèvent à 1,2 million. Malgré des progrès dans la lutte contre la maladie, le nombre de cas de tuberculose reste très élevé, à 10,4 millions par an, dont 1 million de cas pédiatriques. La tuberculose est une cause majeure de mortalité chez les enfants infectés par le VIH, représentant plus d'un tiers des décès chez ceux-ci. Il n'existe actuellement pas d'estimation précise de la prévalence du VIH dans le million de cas de tuberculose pédiatrique survenant chaque année. Cependant, les décès liés au VIH pourraient représenter jusqu'à 1/5 de tous les décès dus à la tuberculose chez les enfants. Les enfants infectés par le VIH ont un risque beaucoup plus élevé de développer une tuberculose maladie lorsqu'ils sont infectés, du fait de l'immunodépression cellulaire.

Le diagnostic de tuberculose est difficile chez les enfants du fait de la nature paucibacillaire de la maladie et des difficultés de recueil d'échantillons respiratoires. Il constitue un obstacle majeur à l'accès au traitement. Plus de 96% des décès pourraient survenir chez des enfants non traités. Les enfants infectés par le VIH présentent des difficultés diagnostiques accrues et une mortalité plus élevée. Au niveau microbiologique, en plus de la bacilloscopie et de la culture dont la sensibilité est limitée chez les enfants (environ 10 à 15% et 40%, respectivement), le test Xpert MTB/RIF (Xpert), un outil de diagnostic moléculaire simple et automatisé recommandé chez l'enfant en 2013 par l'OMS, a montré une sensibilité de 62% à 66% (crachats induits et/ou expectorés ou aspiration/lavage gastrique) et une spécificité de 98% pour la tuberculose confirmée par la culture. Les méthodes de prélèvements jouent un rôle important chez l'enfant en plus du test microbiologique utilisé. Les méthodes standards recommandées par l'OMS telles que les crachats expectorés ou induits ou l'aspiration gastrique présentent des difficultés techniques. Les crachats expectorés ne peuvent être réalisés en dessous de l'âge de 5 ans et sont peu pratiques entre 5 et 10 ans. L'aspiration gastrique est invasive et nécessite le plus souvent une hospitalisation. Les crachats induits sont peu réalisés en pratique et nécessitent l'ajout d'une aspiration nasopharyngée chez les enfants les plus jeunes. Des prélèvements alternatifs tels que l'aspiration nasopharyngée seule, les prélèvements de selles, ou le string test ont montré leur intérêt et des performances proches des prélèvements standards avec les différents tests microbiologiques. Le rendement de l'Xpert pour la détection de la tuberculose chez l'enfant reste malheureusement faible. De nombreux scores ou algorithmes diagnostiques ont été développés pour pallier à la faible fréquence de la confirmation microbiologique chez l'enfant. La grande majorité des scores et algorithmes ont été développés sur la base d'avis d'experts, avant l'épidémie de VIH. Deux méta-analyses ont révélé l'hétérogénéité de ces systèmes, leur absence de validation, et leur faible performance chez les enfants infectés par le VIH. Aucun score ou algorithme n'est actuellement recommandé par l'OMS.

La tuberculose est plus grave et le risque de décès plus élevé chez les enfants infectés par le VIH par rapport aux enfants non-infectés. L'accès au traitement antirétroviral a entraîné une réduction de l'incidence de la tuberculose dans les cohortes d'enfants infectés par le VIH. L'incidence reste néanmoins plus élevée que chez les enfants non infectés. Une double décision thérapeutique doit être prise chez les enfants infectés par le VIH présentant une suspicion de tuberculose. Le traitement antituberculeux à base de 4 médicaments (isoniazide, rifampicine, éthambutol et pyrazinamide) doit être débuté pour une durée de 6 mois le plus rapidement possible en cas de tuberculose. Un traitement antirétroviral est maintenant recommandé chez tous les enfants. Les recommandations actuelles d'introduction du traitement antirétroviral dans les 2 mois suivant le début du traitement

antituberculeux, et dans les 2 semaines en cas d'immunodépression profonde, sont basées sur des données provenant d'essais cliniques chez des adultes. Le risque de syndrome inflammatoire de restauration immunitaire est faible mais non négligeable chez l'enfant en comparaison avec l'adulte.

Le but de cette thèse était de contribuer à l'amélioration du diagnostic de la tuberculose chez les enfants infectés par le VIH par le développement d'un algorithme diagnostic. L'ensemble des travaux de cette thèse ont été réalisés sur l'étude ANRS 12229 PAANTHER 01 dont j'ai été l'investigateur coordinateur depuis 2010. L'étude ANRS 12229 PAANTHER 01 était une étude de cohorte multicentrique mise en œuvre d'avril 2011 à décembre 2014 dans 8 hôpitaux au Burkina Faso, Cambodge, Cameroun et Vietnam. Son objectif principal était de développer un algorithme pour améliorer le diagnostic de la tuberculose chez les enfants infectés par le VIH dans des pays en développement à forte endémie tuberculeuse. L'étude a inclus 438 enfants d'âge ≤ 13 ans infectés par le VIH avec une suspicion de tuberculose basée sur au moins un des signes suivants : 1) toux persistante depuis plus de deux semaines ; 2) fièvre persistante depuis plus de deux semaines ; 3) retard pondéral ; 4) échec d'un traitement antibiotique à large spectre pour une infection pulmonaire ; 5) anomalie de la radiographie pulmonaire évocatrice de tuberculose.

Après une présentation générale sur la co-infection tuberculose/VIH chez l'enfant, la thèse comprend une série de publications issues de l'étude PAANTHER 01 sur : 1) la mortalité et ses déterminants chez les enfants infectés par le VIH présentant une suspicion de tuberculose ; 2) les performances du test Xpert pour le diagnostic de la tuberculose sur des prélèvements bactériologiques alternatifs ; 3) une étude sur l'accord inter-lecteurs et la valeur diagnostique de la radiographie pulmonaire pour le diagnostic de tuberculose chez les enfants infectés par le VIH ; 4) le développement d'un score diagnostique et d'un algorithme qui était objectif principal de l'étude PAANTHER 01. Des données sur l'utilisation des définitions de cas cliniques standardisées proposées par des experts internationaux comme diagnostic de référence pour les études diagnostique sur la tuberculose chez les enfants et les problèmes méthodologiques rencontrés dans la conduite et l'analyse de ces études sont discutés. Je conclurai en discutant des perspectives pour de futures recherches sur le diagnostic de la tuberculose infantile.

3. La mortalité et ses déterminants chez les enfants infectés par le VIH suspects de tuberculose, une étude de cohorte observationnelle

Contexte : La tuberculose est une cause majeure de morbi-mortalité chez les enfants infectés par le VIH, mais elle est difficile à diagnostiquer. Nous avons étudié la mortalité et ses déterminants chez des enfants infectés par le VIH naïfs de traitement antirétroviral (ARV) présentant une suspicion de tuberculose.

Méthodes : Des enfants infectés par le VIH âgés de ≤ 13 ans avec une suspicion de tuberculose ont été suivis 6 mois dans la cohorte ANRS 12229 PAANTHER 01 (Burkina Faso, Cambodge, Cameroun, Vietnam). Les enfants ont commencé un traitement antirétroviral et/ou antituberculeux à l'appréciation du clinicien local et ont été classés rétrospectivement en 3 groupes selon la documentation sur la tuberculose (confirmée par la culture ou l'Xpert MTB/RIF, non confirmée et improbable). Nous avons évalué la mortalité et ses facteurs associés en utilisant la méthode de Kaplan-Meier et des modèles de Cox à risques proportionnels.

Résultats : 266 enfants naïfs d'antirétroviraux (tuberculose confirmée, $n = 40$, tuberculose non confirmée, $n = 119$, tuberculose improbable, $n = 107$) ont été suivis pendant 112,5 personne-années. 154 (57,9%) ont débuté un traitement antituberculeux et 212 (80,9%) ont commencé un traitement antirétroviral ; 50 (19,5%) enfants sont morts. La mortalité était significativement plus élevée chez les enfants ayant une tuberculose confirmée (14 décès) que chez ceux ayant une tuberculose non confirmée (19 décès) et une tuberculose improbable (17 décès) (log rank=0,014), et plus faible chez

les enfants atteints de tuberculose confirmée ou non confirmée ayant débuté un traitement antituberculeux ($p < 0,0001$ et $< 0,0001$). En analyse multivariée, le traitement antirétroviral débuté au cours du premier mois de suivi [risque relatif (RR) 0,084 ; IC 95% 0,010-0,670], la tuberculose confirmée, le jeune âge, des CD4 $< 10\%$, une miliaire à la radiographie, et des taux élevés de transaminases étaient tous indépendamment associés à la mortalité.

Conclusion : La mortalité était élevée au cours des six premiers mois suivant la suspicion de tuberculose chez les enfants n'ayant jamais reçu de traitement antirétroviral. Le traitement antirétroviral devrait être introduit rapidement, en particulier chez les enfants présentant des facteurs associés à une mortalité élevée. La décision documentée ou empirique de traitement de la tuberculose doit être accélérée pour réduire la mortalité et permettre l'introduction précoce du traitement antirétroviral.

4. Performance du test Xpert MTB/RIF et méthodes alternatives de prélèvement d'échantillons pour le diagnostic de la tuberculose chez les enfants infectés par le VIH

Contexte : Le diagnostic de la tuberculose chez les enfants infectés par le VIH est difficile. Nous avons évalué la performance des méthodes alternatives de prélèvement d'échantillons pour le diagnostic de la tuberculose chez les enfants infectés par le VIH, en utilisant le test Xpert MTB/RIF (Xpert).

Méthodes : Des enfants infectés par le VIH âgés de ≤ 13 ans présentant une suspicion de tuberculose intra-thoracique ont été recrutés dans 8 hôpitaux au Burkina Faso, Cambodge, Cameroun et Vietnam. Les enfants bénéficiaient d'échantillons standards comprenant des aspirations gastriques, s'ils étaient âgés de moins de 10 ans, ou des prélèvements de crachats expectorés, s'ils étaient âgés de 10 ans ou plus, et d'échantillons alternatifs comprenant aspiration nasopharyngée et selles chez tous les enfants, et « string test » chez les enfants de 4 ans et plus. Tous les échantillons ont été testés avec l'Xpert. Nous avons analysé l'exactitude diagnostique de l'Xpert sur diverses combinaisons d'échantillons pour la tuberculose confirmée par culture dans une approche en intention-de-diagnostiquer et per protocole.

Résultats : Sur 281 enfants inclus, 272 (96,8%) avaient ≥ 1 échantillon testé par Xpert (population en intention-de-diagnostiquer). Leur âge médian était de 7,2 ans, les CD4 médian de 14,5%. 179 (63,5%) enfants avaient tous les échantillons et l'Xpert réalisé selon le protocole (population per protocole). La tuberculose a été confirmée par la culture chez 29/272 enfants (10,7%). Les sensibilités en intention-de-diagnostiquer de l'Xpert effectuées sur tous les échantillons, sur les échantillons standard et sur les échantillons alternatifs étaient de 79,3% (IC 95%: 60,3-92,0), 72,4% (52,8-87,3) et 75,9% (56,5-89,7), respectivement. Les spécificités étaient toutes $\geq 97,5\%$. L'Xpert réalisé sur un string test avait une sensibilité en intention-de-diagnostiquer et en per protocole de 36,0% (18,0-57,5) et de 69,2% (30,8-89,1), respectivement. L'Xpert réalisé sur la combinaison aspiration rhinopharyngée et selles avaient une sensibilité en intention-de-diagnostiquer et en per protocole de 75,9% (56,5-89,7) et de 75,0% (47,6-92,7), respectivement.

Conclusion : La combinaison d'une aspiration nasopharyngée et d'un échantillon de selles est une alternative prometteuse aux méthodes de collecte d'échantillons bactériologique habituellement recommandées par les programmes nationaux. L'Xpert effectué sur des échantillons respiratoires et des selles permet une confirmation rapide du diagnostic de la tuberculose chez les enfants infectés par le VIH.

5. Valeur de la radiographie thoracique dans le diagnostic de tuberculose chez les enfants infectés par le VIH vivant dans des pays à ressources limitées. Résultats de l'étude ANRS 12229 - PAANTHER 01

Objectif : Dans cette étude, notre objectif était d'évaluer la reproductibilité inter-lecteur et la validité diagnostique de la radiographie thoracique standard dans le diagnostic de la tuberculose chez les enfants infectés par le VIH.

Méthodes : Une étude prospective a été menée au Burkina Faso, au Cambodge, au Cameroun et au Vietnam d'avril 2010 à mai 2014. Des enfants infectés par le VIH et âgés de ≤ 13 ans avec une suspicion clinique de tuberculose ont été recrutés. Trois lecteurs, y compris des radiologues locaux (RL), un pneumologue pédiatrique (PP) et un radiologue pédiatrique (RP) ont revu indépendamment chaque radiographie, en insu d'autres informations cliniques et biologiques. L'accord a été évalué sur la présence et la localisation de lésions radiographiques compatibles avec la tuberculose : opacité alvéolaire, excavation, foyer de Ghon, miliaire, infiltrat nodulaire, adénopathies para-trachéales ou péri-hilaires, compression trachéale ou bronchique, épanchement pleural, gibbus. Nous avons évalué l'exactitude diagnostique de la radiographie pulmonaire après consensus final dans une analyse cas-témoins (tuberculose confirmée par la culture Vs tuberculose improbable suivie sans traitement sans traitement antituberculeux à 6 mois).

Résultats : 403 enfants (âge médian de 7,3 ans [IQR: 3,5-9,7], 49,6% de garçons) ont été inclus dans l'étude. L'accord entre lecteurs sur les lésions compatibles avec la tuberculose, exprimé par le coefficient Kappa, était le suivant : RL versus PP: $K = 0,36$ [IC 95% 0,27-0,45]; RL Vs. RP 0,16 [IC 95% 0,08-0,24]; PP Vs. RP 0,30 [IC 95% 0,21-0,40]. Parmi les 51 cas et les 151 témoins, les radiographies après consensus définitif avaient une sensibilité de 71,4% [IC 95% 58,8-84,1] et une spécificité de 50,0% [IC 95% 41,9-58,1] et une précision diagnostique globale de 55,3%.

Conclusion : L'analyse de la radiographie thoracique a montré une concordance inter-lecteurs mauvaise à passable et une précision diagnostique limitée pour la tuberculose chez les enfants infectés par le VIH, probablement en raison de la fréquence des comorbidités, alors qu'un diagnostic précis et rapide est nécessaire pour réduire la mortalité tuberculeuse. Les critères radiologiques doivent être discutés pour cette population spécifique.

6. Un score de prédiction diagnostique et un algorithme de décision de traitement antituberculeux pour les enfants infectés par le VIH avec une suspicion de tuberculose.

Contexte : Le diagnostic de la tuberculose chez les enfants infectés par le VIH devrait être amélioré afin de raccourcir le délai avant le début du traitement antituberculeux et du traitement antirétroviral et de réduire la mortalité. Nous avons développé des scores de prédiction diagnostique pour la décision de traitement antituberculeux chez les enfants infectés par le VIH avec une suspicion de tuberculose.

Méthodes : Des enfants infectés par le VIH avec une suspicion de tuberculose ont été inclus dans l'étude ANRS 12229 PAANTHER 01 au Burkina Faso, au Cambodge, au Cameroun et au Vietnam. Les enfants ont eu une évaluation clinique, une radiographie thoracique, un test Quantiferon Gold In-Tube (QFT), une échographie abdominale, des prélèvements d'échantillons respiratoires et de selles pour la réalisation d'examen de microbiologie incluant le test Xpert MTB/RIF (Xpert). Nous avons développé 4 modèles diagnostiques de tuberculose (confirmée ou non confirmée) par régression logistique : 1) incluant tous les prédicteurs, 2) excluant le QFT, 3) excluant l'échographie, 4) excluant le QFT et l'échographie abdominale. Nous avons secondairement inclus les résultats de microbiologie dans les modèles. Nous avons construit des scores basés sur les modèles avec la meilleure aire sous la courbe de ROC (AUROC) et des seuils de probabilité individuelle prédite obtenant une sensibilité $>90\%$ chez les tuberculoses confirmées.

Résultats : 438 enfants ont été inclus dans l'étude avec un âge médian de 7,3 ans, 220 (50,2%) de sexe masculin, 172 (39,3%) sous traitement antirétroviral à l'inclusion. 251 (57,3%) enfants avaient une tuberculose [55 (12,6%) tuberculoses confirmées par la culture ou l'Xpert et 196 (44,7%) non

confirmée]. Les 4 modèles développés chez 335 enfants avec tous les prédicteurs disponibles incluaient : la fièvre > 2 semaines, la toux persistante, une hémoptysie et une perte de poids au cours des 4 dernières semaines, un contact avec un cas de tuberculose à bacilloscopie positive, une tachycardie, la miliaire, des opacités alvéolaires et des adénopathies sur la radiographie thoracique, des adénopathies abdominales à l'échographie, et les résultats du QFT. Les AUROC étaient de 0,839, 0,830, 0,819, 0,808 et 0,866, 0,861, 0,850, 0,846 en incluant Xpert, pour les modèles 1, 2, 3 et 4, respectivement. Les scores développés sur le modèle 2 avec et sans Xpert avaient une sensibilité de 91,5% ou 88,6% (avec Xpert) et une spécificité de 42,5% ou 61,2% (avec Xpert) pour le diagnostic de la tuberculose.

Conclusion : Les scores développés ont une bonne performance diagnostique, surtout si l'Xpert est disponible. Utilisés dans un algorithme, ils devraient permettre de prendre rapidement des décisions de traitement chez les enfants présentant une suspicion de tuberculose et un risque de mortalité élevé, contribuant ainsi à des bénéfices significatifs en termes de santé publique.

7. Utilisation de la Définition Clinique des Cas pour la Classification de la Tuberculose Intra-thoracique chez les enfants infectés par le VIH

Objectif : Nous visions à évaluer l'impact de l'utilisation de la Définition Clinique des Cas pour la Classification de la Tuberculose Intra-thoracique actualisée proposée pour la recherche (Graham *et al*, CID, 2015), sur le diagnostic de référence tuberculose des enfants, par rapport à la classification originale (Graham *et al*, JID, 2012).

Méthodes : Nous avons appliqué rétrospectivement la version 2012 et la version 2015 de la Définition Clinique des Cas pour la Classification de la Tuberculose Intra-thoracique à tous les enfants de la cohorte, de façon automatisée. Nous avons évalué l'impact des données manquantes et du fait d'exclure le QFT de la définition des preuves immunologiques de l'infection à MTB sur la classification, l'accord inter-classification pour le diagnostic de tuberculose (tuberculose confirmée et probable Vs. autres catégories pour la version 2012, et tuberculose confirmée et non confirmée Vs. improbable pour la version 2015) et identifié les facteurs associés à l'initiation du traitement de la tuberculose par régression logistique.

Résultats : Sur 438 enfants inclus dans l'étude, 55 (12,6%) avaient une tuberculose confirmée, 196 (44,7%) une tuberculose non confirmée et 187 (42,7%) une tuberculose improbable. Selon la version de 2012 de la définition des cas, 197 (45,0%) enfants étaient classés comme tuberculose alors que 251 enfants étaient considérés comme tuberculose par la version 2015 (accord inter-classification 82,9%, kappa 0,6670 (IC à 95% 0,5999 - 0,7341)). 10 enfants avec tuberculose improbable avaient des données microbiologiques manquantes et pourraient donc avoir été mal classés comme improbables. En considérant tous les radiographies manquantes ou sans conclusion comme positives, 13 (3,0%) enfants supplémentaires ont été reclassés de tuberculose improbable à tuberculose non-confirmée. Considérer les résultats positifs de QFT comme preuve immunologique supplémentaire de l'infection à MTB n'avait aucun impact sur la classification des enfants. 10 enfants avaient un Xpert positif sans culture positive dont 4 tuberculoses possibles, et 3 improbables ou non-tuberculose selon la classification 2012. 10 avec une tuberculose probable (2012) n'ont pas été considéré comme une tuberculose par la classification 2015 malgré la présence de symptômes, lésions radiographiques et contact ou critère de LTBI. 58 enfants tuberculose probable ont été reclassés en tuberculose du fait que 2 critères seulement sont nécessaires pour classer comme tuberculose non confirmée s'il n'y a pas d'amélioration spontanée sans traitement antituberculeux. Les facteurs indépendamment associés à l'initiation du traitement antituberculeux étaient le pays, les antécédents de tuberculose, une

radiographie pulmonaire compatible avec la tuberculose, des résultats d'Xpert et/ou de bacilloscopie positifs, une fièvre >2 semaines et un score de Lansky <90.

Conclusion : L'accord entre les classifications 2012 et 2015 était bon. La classification dépend fortement de la décision du clinicien de traiter et peut donc manquer de spécificité, selon les pratiques cliniques du site.

8. Discussion générale/9. Conclusion

Malgré des limites méthodologiques essentiellement liées à l'absence de gold standard chez l'enfant, et donc à l'utilisation d'un standard de référence composite et aux problèmes qui en découlent tels qu'un biais d'incorporation et de la vérification différentielle, ce travail souligne la nécessité d'un diagnostic rapide de la tuberculose chez les enfants infectés par le VIH afin de réduire la mortalité. Il propose d'améliorer le diagnostic par une approche alternative à la confirmation microbiologique et le développement d'un algorithme rapide de décision de traitement chez les enfants à haut risque de mortalité. Il met en évidence les défis pratiques et méthodologiques liés à l'utilisation de la radiographie pulmonaire et du standard de référence diagnostique dans cette population. Le projet TB-Speed permettra la validation externe de l'algorithme chez les enfants infectés par le VIH et contribuera à l'amélioration du diagnostic de la tuberculose et à la réduction de la mortalité chez les enfants en décentralisant la confirmation microbiologique et le diagnostic dans des pays à faible revenu.

List of personal publications and scientific communications on the topic

- Articles in peer-reviewed journals

Published

Marcy O, Ung V, Goyet S, Borand L, Msellati P, Tejiokem M, Nguyen Thi NL, Nacro B, Cheng S, Eyangoh S, Pham TH, Ouedraogo AS, Tarantola A, Godreuil S, Blanche S, and Delacourt C. for the PAANTHER study group. Performance of Xpert MTB/RIF and alternative specimen collection methods for the diagnosis of tuberculosis in HIV-infected children. Clin Infect Dis. 2016 May 1;62(9):1161-8.

In press

Marcy O, Tejiokem M, Msellati P, Truong HK, Do CV, Tran ND, MD, Nacro B, Ateba-Ndong F, Tetang-Ndiang S, Vibol Ung V, Dim B, Neou L, Berteloot L, Borand L, Delacourt C, Blanche S, for the ANRS 12229 PAANTHER 01 Study Group. Mortality and its determinants in ART-naïve HIV-infected children with a suspicion of tuberculosis, an observational cohort study. Lancet HIV. In press.

Submitted

Marcy O, Borand L, Ung V, Msellati P, Tejiokem M, Truong HK, Do CV, Tran ND, Ateba-Ndong F, Tetang-Ndiang S, Nacro B, Neou L, Goyet S, Dim B, Quillet C, Fournier-Nicollé I, Berteloot L, Carcelain G, Godreuil S, Blanche S, Delacourt C, for the ANRS 12229 PAANTHER 01 Study Group. A diagnostic prediction score for tuberculosis treatment decision in HIV-infected children with a suspicion of tuberculosis.

Berteloot L, **Marcy O**, Nguyen B, Ung V, Tejiokem M, Nacro B, Goyet S, Dim B, Blanche S, Borand L, Msellati P, Delacourt C. Value of Chest X Ray in the diagnosis of tuberculosis in HIV-infected children living in resource limited countries. Results from the ANRS 12229 - PAANTHER 01 study.

- Scientific communications

Invited oral communication

Marcy O, Borand L, Tejiokem M, Ateba-Ndong F, Truong HK, Nacro B, Msellati P, Ung V, for the ANRS 12 229 PAANTHER 01 Study Team. Morbidity and mortality in TB-HIV co-infected children on TB treatment initiated on ART in the PAANTHER cohort in Asia and West Africa. 46th Union Conference on Lung Health, Cape Town, South Africa, 2-6 December 2015.

Oral communication

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par le VIH. 7ème conférence internationale francophone VIH/hépatites, AFRAVIH, Montpellier, 23-27 avril 2014.

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Poster

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Marcy O, Tejiokem M, Msellati P, Truong HK, Nacro B, Ateba-Ndong F, Vibol U, Bunnet D, Borand L, Delacourt C, Blanche S. Mortality and associated factors in ART-naïve HIV-infected children with a suspicion of tuberculosis. 21st International AIDS Conference (AIDS 2016) Durban, South Africa | July 18-22, 2016.

Marcy O, Tejiokem M, Msellati P, Truong HK, Nacro B, Ateba-Ndong F, Vibol U, Bunnet D, Borand L, Delacourt C, Blanche S. Mortality and associated factors in ART-naïve HIV-infected children with a suspicion of tuberculosis. TB 2016, 16-17 July 2016, Durban, South Africa

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1 Introduction

Tuberculosis is now the leading infectious cause of death worldwide, with an estimated mortality of 1.8 million deaths per year, including 400,000 in HIV-infected individuals, when HIV deaths sum up to 1.2 million (1). Despite progress in reducing tuberculosis incidence and mortality since 1990, morbi-mortality remains unacceptably high at 10.4 million cases every year. The estimated 17% case fatality rate is largely due to poor case detection, with only 2/3 of adult cases notified and only 1/3 of multidrug resistant (MDR) tuberculosis properly detected and treated every year.

The Sustainable Development Goals (SDGs) for 2030 were adopted by the United Nations in 2015. One of the targets is to end the global tuberculosis epidemic. The WHO End TB Strategy, approved by the World Health Assembly in 2014, calls for a 90% reduction in TB deaths and an 80% reduction in the TB incidence rate by 2030, compared with 2015.

Childhood tuberculosis has long been a neglected disease (2, 3). Thought to be less infectious and therefore contributing only minimally to onward transmission, childhood tuberculosis was not a priority for national tuberculosis programs and for the DOTS strategy, launched by the WHO in 1993, which was mostly based on the detection and treatment of smear-positive cases (4). This was not taking into account the major morbi-mortality suffered by children with tuberculosis. With 1 million tuberculosis cases every year and a case-fatality estimated between 20 and 24% (1, 5), children pay a heavy tribute to the disease. There have been massive efforts in the past 10 to 15 years to put childhood tuberculosis on the top of the public health agenda (6), resulting in major advances and the first publication by WHO of specific pediatric guidance for national tuberculosis programs in 2006, updated in 2014 (7, 8). A “Roadmap for Childhood Tuberculosis”, endorsed by multiple international institutions and agencies has identified major gaps and steps to take toward the goal of “zero TB deaths in children” (9). This includes improving childhood tuberculosis diagnosis which presents major challenges due to the difficulties in obtaining microbiological confirmation, unlike in adults. Lack of proper diagnosis is the major barrier to treatment of this curable disease (10). Recent estimates showed that more than 96% of tuberculosis deaths in children could be due to the lack of treatment (5).

Despite limited large scale epidemiological data and estimates on tuberculosis and HIV co-infection in children, there is evidence that tuberculosis is one of the major opportunistic infections in HIV-infected children. As in adults, tuberculosis accounts for 1/3 of causes of death in children living with HIV. Diagnostic challenges are even greater in HIV-infected children in a context of higher mortality (11, 12). New tools for diagnosis and treatment decision in HIV-infected children presenting with a suspicion of tuberculosis need to be evaluated and integrated into a global diagnostic approach.

My PhD thesis objective is to develop an algorithm for the diagnosis of tuberculosis in HIV-infected children in developing countries with a high tuberculosis burden and thus contribute to improving diagnosis of tuberculosis in HIV-infected children. It is based on the ANRS 12229 PAANTHER 01 study, which I conducted as coordinating investigator. The PAANTHER 01 study was implemented in two African countries (Burkina Faso and Cameroon) and two South-East Asian countries (Cambodia and Vietnam) between 2011 and 2014.

After a general presentation on tuberculosis and HIV co-infection in children, I will present a series of publications issued from the PAANTHER 01 study on: 1) mortality and its determinants, including diagnostic factors, in ART-naïve HIV-infected children with a suspicion of tuberculosis; 2) performances of Xpert MTB/RIF (Xpert), an automated and simple molecular diagnostic tool, for tuberculosis diagnosis on alternative bacteriological samples easier to perform than samples methods currently recommended by the WHO; 3) inter-reader agreement and diagnostic value of chest radiography for tuberculosis diagnosis in HIV-infected children from low-income countries; 4) and

finally, the diagnostic score and algorithm developed as the primary objective of the study. I will also present data on the use of standardized clinical case definitions proposed by international experts as reference standards for tuberculosis diagnostic studies in children and discuss some of the methodological issues I encountered in conducting and analyzing those studies. I will conclude by discussing the perspectives for future research on childhood tuberculosis diagnosis.

2 Tuberculosis and HIV co-infection in children

2.1 Pathogenesis of tuberculosis and tuberculosis/HIV co-infection

Tuberculosis is a communicable airborne infectious disease caused by mycobacteria from the *Mycobacterium tuberculosis* (MTB) complex (13). Mycobacteria are aerobic nonmotile bacilli. Compared to other bacteria, they are characterized by a hydrophobic and thick cell wall which makes them hard to stain with usual bacteriological dyes such as the Gram stain, and is not easily decolorized when using specific colorations (Ziehl-Neelsen staining) (14). They are thus referred to as acid-fast or acid/alcohol-fast. The mycobacteria genus includes mycobacteria from the MTB complex, non-tuberculous, or atypical, mycobacteria (NTM), as well as *M. leprae* and *M. ulcerans*, the causing agents of leprosy and the Buruli ulcer. *M. tuberculosis* is the most frequent mycobacteria from the MTB complex involved in human pathology. Its reservoir is humans. Other bacteria from the MTB complex include *M. bovis*, *M. africanum*, *M. microti*, *M. caprae*, *M. pinnipedii*, *M. canetti*, and *M. mungi*. *M. tuberculosis* organisms are also called tubercle bacilli or Koch's bacilli.

Tuberculosis transmission is almost invariably occurring via the respiratory tract. Bacilli included in microscopic airborne particles (droplet nuclei) exhaled by tuberculosis cases through cough are inhaled by children or adults. This can occur in the family environment but outbreaks can occur in almost any setting, such as school or work environment and public transportation (13, 15). Inhaled bacilli are ingested by macrophages in the alveoli which results, through a series of complex immunological mechanism, in either i) complete elimination of the bacilli; ii) persistence of the bacilli in dormancy state usually in a granuloma, known as latent tuberculosis infection (LTBI) or iii) tuberculosis disease resulting from poor containment of the primary infection, usually due to immune deficiency or in young children. In adults and adolescents, the disease can also occur secondarily from the progression from LTBI. The prevalence of LTBI has been re-estimated recently by mathematical modelling to 23.0% (95% uncertainty interval [UI]: 20.4%-26.4%) of the total world population, i.e. approximately 1.7 billion people (16). It is now increasingly recognized that there is a continuum between tuberculosis exposure, infection, subclinical disease, non-severe disease and severe disease (17).

Cell-mediated immune responses, specifically those mediated by CD4⁺ T cells, contribute to the control of both HIV and TB. Thus, HIV-infected individuals, whose CD4⁺ T cells are depleted, are less capable of controlling replication of MTB. They are more likely to progress rapidly from primary infection to tuberculosis disease and develop re-activation of LTBI. After infection with MTB via the respiratory tract, alveolar macrophages present mycobacterial antigens to CD4⁺ T cells. This results in the release of interferon- γ and other cytokines, which in turn activates macrophages to control MTB infection. Without the recruitment and activation of macrophages by the CD4⁺ T-cell secreted cytokines in HIV-positive individuals, there is poor granuloma formation, little or absent caseous necrosis, poor containment of mycobacteria with large organism loads and hematogenous dissemination (18). Conversely, MTB infection supports HIV-1 replication and dissemination through the dysregulation of host cytokines, chemokines, and their receptors (19). Development of tuberculosis in HIV-infected individuals without severe immunodeficiency appears to increase the rate of HIV disease progression and mortality (20). Localized immune stimulation by tuberculosis may lead to higher HIV viral loads, increased HIV-1 diversity, and changes in cytokine/chemokine levels in HIV-infected individuals with tuberculosis. Specifically, tuberculosis induced increase in cytokine levels, such as TNF α and MCP-1, may activate HIV replication in lymphocytes, monocytes, and macrophages in organs infected by MTB.

It is estimated that globally, 5% of primary infection result in tuberculosis, and that people with LTBI have an additional lifelong individual risk of developing tuberculosis disease of approximately 5% (21). In HIV-infected individuals and young children, who have immature immune systems, the risk of tuberculosis disease is much higher. A study showed that adults living with HIV co-infected with tuberculosis have a 5–15% annual risk and a 50–60% lifetime risk of developing tuberculosis disease compared with HIV-uninfected individuals, whose lifetime risk is only of 10% (22). The risk of progression to tuberculosis disease following infection in children below the age of one year has been estimated to 50%, with a very high proportion of disseminated and severe forms of the disease, including tuberculous meningitis and miliary disease (23). The risk decreases significantly in the second year of life onward and is the lowest between the ages of 5 to 10 years. During adolescence, the risk of developing adult-type disease is estimated to 10–20%. This can occur from primary infection, endogenous reactivation or exogenous reinfection. This increased risk in adolescents is poorly understood. HIV-infected children with LTBI are more likely to develop tuberculosis disease (24, 25), with a potentially rapidly evolutive disease. A recent study in South Africa reported a 24-fold increase in the risk of developing culture-confirmed tuberculosis in HIV-infected infants compared with HIV-negative infants (25). HIV-infected children may present more acute forms with more severe prognosis and higher mortality (26).

Pulmonary tuberculosis is the most common form of tuberculosis in HIV-infected children (27). Studies have contradictory findings regarding the impact of HIV-infection on the proportion of extra-pulmonary forms, with some cohorts reporting similar rates than in non-HIV infected children and others reporting very high rates of extra-thoracic disease. Various reports show that intra-thoracic and extra-thoracic disease occurs jointly in 22% of cases and that disseminated disease accounts for 11–16% (28–30). As in non-HIV infected children, tuberculosis is paucibacillary in HIV-infected children, compared to adult-type disease. As an example, a 2-cm large cavity, typical feature of adult-type disease, can contain up to 10^7 to 10^9 bacilli (31), when a tuberculous nodule of the same size, closer to pediatric forms of the disease, contains only 10^2 to 10^4 bacilli (32).

2.2 Epidemiology of tuberculosis in HIV-infected children

In 2015, the WHO estimated the annual burden of tuberculosis to 10.4 million incident cases worldwide, including 1.2 million (11%) cases in people living with HIV (1). With an estimated total of 1.8 million tuberculosis deaths in 2015, including 0.4 million tuberculosis-associated deaths in people living with HIV, tuberculosis remained one of the ten first causes of death worldwide in 2015, and one of the two lead infectious cause of death with HIV (33, 34).

In 2012, the WHO estimated for the first time the burden of pediatric tuberculosis in its annual tuberculosis report. Using methods similar to those used in adults based on extrapolation from the number of notified cases, the number of new tuberculosis cases in children was estimated to 0.5 million cases in 2011 and the number of tuberculosis related deaths in children to 64,000 (35). In line with recent mechanistic modelling work, the WHO revised its estimates in 2015; it was estimated that there were 1.0 million new tuberculosis cases in children worldwide (36). Unlike adults where 60% of cases are notified to the WHO, in 2014, only 36% (359,000 cases) of childhood tuberculosis cases were notified. This is due mainly to major challenges in the diagnosis of the disease in children, as well as reporting challenges in countries with large proportions of tuberculosis treated in the private sector, such as India (37). There were an estimated 210,000 to 239,000 deaths attributed to tuberculosis in children in 2015, resulting mostly from lack of diagnosis and access to treatment (1, 5). Indeed, data from large tuberculosis pediatric cohorts show that, with access to treatment, the pooled case fatality ratio dropped from 21.9% (95% CI 18.1–26.4) during the pre-treatment era to 0.9% (95% CI 0.5–1.6) after 1980 (10). In children below the age of 5 the pooled case fatality ratio

was much higher, at 43.6% (95% CI 36.8–50.6), and dropped down at 2.0% (95% CI 0.5–7.4) with the introduction of tuberculosis treatment. Based on these pooled case fatality ratios and other inputs, a recent mathematical modelling study estimated that more than 96% of the 239,000 tuberculosis death in children occurred in children not receiving tuberculosis treatment (5).

It is estimated that 650,000 to 840,000 childhood tuberculosis cases occur in 22 countries identified as high-burden countries by the WHO (36). The list of high-burden countries, as well as the lists of countries with high tuberculosis-HIV co-infection and those with high drug-resistant tuberculosis burden, have been updated in 2015 (1). They now include 30 countries. India itself represents 26% of all pediatric tuberculosis cases.

They were an estimated 2.1 million children living with HIV in 2016, with 160,000 new HIV infections in children (38). Mortality due to AIDS-related illnesses in children decreased from 210,000 deaths in 2010 to 120,000 deaths in 2016. This decline is due to a reduction in the number of new pediatric HIV-infections as well as increased access to pediatric antiretroviral therapy (ART). Indeed there have been major recent progresses in access to ART for children with estimated ART coverage increasing from 34% in 2013 to 52% in 2016 (38). Access to ART has been shown to lead, as in adults, to major reduction in the incidence of opportunistic infections in children living with HIV.

There are currently no real estimates of the burden of tuberculosis-HIV co-infection in children. Estimates through mechanistic modelling have determined that the overall prevalence of HIV-infection among tuberculosis cases in children and adolescents could be around 5% overall, and reach 25% in countries such as South-Africa and Zimbabwe (36). These estimates remain questionable as WHO estimates of total tuberculosis-related deaths in children could amount to 210,000 overall, including 40,000 children living with HIV (1). Tuberculosis remains nonetheless one of the major causes of morbidity and mortality in HIV-infected children worldwide. The 40,000 estimated tuberculosis-related deaths in HIV-infected children represent 17% of all childhood tuberculosis deaths and 1/3 of the 120,000 deaths in HIV-infected children (5, 39). This situation is likely to continue, as ART coverage barely exceeds half of the overall pediatric HIV infected population. Furthermore, as in recent reports in adults, children failing ART may secondarily present with tuberculosis.

2.3 Diagnosis of tuberculosis in HIV-infected children

Suspicion of tuberculosis in children living with HIV is therefore a frequent situation. The WHO recommends investigating for tuberculosis all HIV-infected children presenting with poor weight gain, fever, current cough or history of contact with a tuberculosis case (40).

2.3.1 Microbiology

The diagnosis of tuberculosis in children is complicated by the absence of a laboratory gold standard. Bacteriological confirmation is rarely achieved, due to the paucibacillary nature of childhood tuberculosis, to the difficulty to expectorate in children, and challenges in bacteriological specimen collection (37, 41).

Microbiological tests to diagnose tuberculosis

Acid-fast staining and smear microscopy

Smear microscopy is the oldest microbiological test for tuberculosis. Rapid, and inexpensive, it has been the cornerstone of the DOTs strategy, and is still in use in many settings. It relies on the direct visualization of the bacilli using standard microscopy or fluorescence/LED microscopes and acid-fast

stains such as the Ziehl-Neelsen staining technique or fluorescent stains (such as Auramine O) (42). The sensitivity of smear microscopy is low with a limit of detection (LOD) of approximately 1000–10,000 colony-forming units (cfu)/mL (43). The sensitivity can be improved slightly by centrifugation and decontamination (44). Due to the low bacillary loads in children, sensitivity of smear microscopy therefore remains low, except in older children which can present adult-type cavitary disease. Acid-fast stains are also not specific for MTB and can detect NTM (45). Overall, sputum smear microscopy is positive in only 10 to 15% of children with probable tuberculosis (46).

Culture and identification

Mycobacterial culture is considered the reference standard in diagnosis of tuberculosis in adults (13). It consists in growing viable bacilli on solid or liquid media after decontamination, followed by identification to distinguish MTB from NTM. The automated Mycobacterial Growth Indicator Tube (Bactec MGIT, Becton Dickinson Microbiology Systems, Cockeysville, Maryland), has progressively replaced culture on solid media such as the Lowenstein Jensen (LJ) media due to its better sensitivity, shorter time to positive and negative results, and improved reproducibility (47). The LOD for culture is approximately of 10 to 100 cfu/mL. Mean time to study results has been reduced from weeks to days by the replacement of solid media by liquid media. Time to final negative results is nevertheless still long, with usual definitive negative results given in 56 days (8 weeks) for LJ cultures, and 422 days (6 weeks) for MGIT culture.

To confirm presence of MTB and differentiate it from NTM, identification methods have to be used. Traditional methods, such as the use of para-nitrobenzoic acid or the niacin test are simple but they require sub-culture to obtain pure isolates which further delays results by 2 to 3 weeks (48). Molecular methods can be used but rapid tests for the detection of the MPT64 antigen are less expensive, easier to implement and provide results within a few hours (48, 49). Access to mycobacterial culture remains very limited in low income settings (50) and culture yields are usually low in children (30 to 40 %) (51, 52). Rate of confirmation varies however between settings and with the severity of disease. In a South African study aiming to assess the effectiveness of ART in reducing incident tuberculosis in HIV-infected children, less than 10% of tuberculosis cases were culture-confirmed (53).

Nucleic Acid Amplification Tests (NAATs)

A wide range of NAATs have been developed and used for years to detect the MTB genome, from in-house polymerase chain reaction (PCR) to fully automated commercial techniques. These molecular techniques enable to perform a rapid tuberculosis diagnosis and to detect mutations associated to drug-resistance. The few pediatric studies available all showed sensitivity equal to or greater than that of cultures in tuberculosis (54-58). However, estimates of accuracy of in-house NAAT in adults are highly heterogeneous (59). Among 84 studies analyzed, sensitivity varied from 9.4% to 100%, and specificity estimates ranged from 5.6% to 100%. In the meta-regression analysis, the use of IS6110 as a target, and the use of nested PCR methods appeared to be significantly associated with higher diagnostic accuracy. The heterogeneity did not allow assessing pooled estimates of diagnostic accuracy. Regarding commercial NAAT, their sensitivity and specificity in respiratory specimens were highly variable, with sensitivity lower and more inconsistent than specificity (60). The pooled sensitivity was 0.85 (range 0.36-1.00) and the pooled specificity was 0.97 (range 0.54-1.00), with significant heterogeneity for both ($p < .001$). This did not allow recommending commercial NAATs alone as replacement tests for conventional tests for diagnosing pulmonary TB.

The NAAT Xpert MTB/RIF (Xpert; Cepheid, Sunnyvale CA, USA) was a major change in the tuberculosis diagnostic landscape. It enables to both detect MTB and resistance to rifampin in 2 hours. It was endorsed by WHO in 2010 for the diagnosis of HIV-associated and drug-resistant tuberculosis in adults following ground breaking results showing a sensitivity of 98.2% in smear-positive

individuals and 72.5% in smear-negative individuals (61). Repeated tests (3 times) increased the sensitivity up to 90.2% in smear-negative patients. Its specificity was of 99.2% in patients without tuberculosis. It identified RIF-resistant MTB with a sensitivity of 97.6% and a specificity of 98.1% compared to phenotypic drug-susceptibility testing. One of the major strength of the test is that its performance is equivalent on untreated sputum and on decontaminated pellet, thus requiring very little manipulation before testing. Furthermore it has been shown that biohazards are significantly reduced with Xpert MTB/RIF assay preparation compared to smear microscopy slide preparation (62). Its good sensitivity and specificity has been confirmed by subsequent studies in adults and a meta-analysis has determined a pooled sensitivity of 89% [95% Credible Interval (CrI) 85% to 92%] and pooled specificity 99% (95% CrI 98% to 99%) as an initial test replacing smear microscopy (63) over 27 studies. A recent meta-analysis showed its interest for rapid diagnosis of culture-confirmed pulmonary tuberculosis in children with a pooled sensitivities and specificities of Xpert for culture-confirmed tuberculosis of 62% (95% CrI 51–73) and 98% (95% CrI 97–99), respectively, with use of expectorated or induced sputum samples and 66% (95% CrI 51–81) and 98% (95% CrI 96–99), respectively, with use of samples from gastric lavage (64). These findings supported the revised WHO recommendations on scaling-up the use of Xpert in children (8), which was expected to have a limited impact on childhood tuberculosis diagnosis overall, due to the limited yield of culture itself. The recent meta-analysis on use of Xpert MTB/RIF in children showed that there was no difference between HIV-infected and non-infected children in terms of performance of the Xpert MTB/RIF assay (64). Since, Xpert MTB/RIF has been incorporated as a confirmatory test in diagnostic reference standards for research, at the same level as culture (65). The Xpert MTB/RIF Ultra

Urinary Lipoarabinomannan

Detection of the mycobacterial cell-wall antigen lipoarabinomannan (LAM) by urine assays has been evaluated recently in the screening and diagnosis of tuberculosis and has shown its interest in HIV-infected individuals, especially when severely immuno-compromised (66-68). It is now recommended for tuberculosis diagnosis for severely ill or hospitalized HIV-infected adults by the WHO (69, 70). In children however, it showed disappointing sensitivity and specificity (71). A recent study in 165 hospitalized HIV-infected Kenyan children with median CD4% 14.3 showed a sensitivity of 43% and a specificity of 91% for the LAM tests(72), consistent with its mediocre performance in adults (69).

Microbiological tests for drug resistance

Drug resistance testing is fundamental to adapt tuberculosis treatment to the resistance pattern of the bacilli. It is estimated that there were globally 480,000 cases of new tuberculosis cases in 2015 which were multi-drug resistant i.e. resistant to the two main drugs of the tuberculosis treatment regimen, rifampin and isoniazid, and an additional 100,000 rifampin resistant cases, for approximately 5.6% of the overall tuberculosis case load that would require 2nd tuberculosis drugs (1). Drug resistance testing includes culture-based phenotypic drug-susceptibility testing (DST) methods which provide a measure of the susceptibility of bacteria to a drug (73). DST was developed on solid media and is now performed on liquid media, with a shorter turnaround time. Drug resistance testing also includes genotypic tests which predict resistance to a drug rather than establish susceptibility (a negative genotypic test result is usually reported as no resistance detected). Drug resistance can arise from mutations in the MTB DNA that render the organism immune to the action of the drug. Xpert MTB/RIF can detect mutations in the rpo B gene involved in more than 95% of resistance to rifampin (61). The WHO has also endorsed Line Probe Assays which are another molecular technology to detect mutations associated to resistance. The most widely studied tests are the GenoType MTBDRplus for rifampin and isoniazid and the GenoType MTBDRsl-v1.0 assay to detect resistance to fluoroquinolones and aminoglycosides, which define extensively drug-resistant (XDR) tuberculosis in addition to resistance to rifampin and isoniazid, and ethambutol.

Bacteriological specimen collection methods

In children, not only the assay but the specimen collection methods, their feasibility and their ability to retrieve bacilli, are key factors contributing to the microbiological diagnosis of tuberculosis. Concerning intra-thoracic tuberculosis, standard collection methods recommended by the WHO include expectorated sputum, gastric aspirates, and induced sputum (8, 52, 74). Alternative sample collection methods which have been tested and are not yet included in formal recommendations from the WHO include nasopharyngeal aspirates, which are a necessary add-on to induced sputum in children too young to expectorate, but can be used as a standalone procedure, without or without nasal instillation, oropharyngeal aspirates, string test, and stool samples. Bronchoalveolar lavage can be used but the technique is highly specialized and not available in most TB endemic areas. For extra-pulmonary tuberculosis, appropriate samples are guided by the localization of the disease and include: pleural tap, cerebro-spinal fluid (CSF) tap, ascites tap, and fine-needle aspirates.

Respiratory samples

Expectorated sputum is the recommended method for respiratory sample collection in adults (75). Sputum collection is not feasible in young children below the age of 5 which are unable to expectorate and is challenging in children between the age of 5 and 10, in whom it can be attempted with appropriate coaching and assistance (43).

Young children, as well as adults, swallow their expectorations which can be collected by gastric aspirates. MTB originating from the respiratory tract reaching the oropharynx through cough or mucociliary clearance can therefore be swallowed and enter the stomach (76). Gastric aspirates should be performed after overnight fasting and are difficult to obtain (7). It usually requires hospitalization although ambulatory gastric aspirates could be as effective (77). Their yield remains low with only 41% of cases confirmed by culture (77).

Induced sputum by hypertonic saline nebulization provides a yield equivalent or superior to gastric aspirates (78, 79). When combined with gastric aspirates, both techniques increase by 50% the number of children with mycobacteria identification (67% with positive culture) (80). This non-invasive method may however pose a risk of nosocomial exposure if no tuberculosis infection control is in place. Nasopharyngeal aspirate by itself, without prior nebulization, may have the same yield as induced sputum (81). Data so far on its performance with Xpert MTB/RIF is limited and did not allow assessing pooled sensitivity and specificity to compare it with that of the gastric aspirate and/or of expectorated/induced sputum (64). At last, bronchoalveolar lavage has performances equivalent to gastric aspirates and has none or little additive value when both techniques are done (54, 82).

The Enterotest® (or string test), previously used for the detection of parasites in the stomach or duodenum, was evaluated in the diagnosis of tuberculosis. This simple technique allows the collection of duodenal or gastric contents on a nylon or cotton string unraveling from a weighted gelatin capsule swallowed by the patient. The end of the string is maintained taped to the cheek during the time required for the string to be coated with stomach fluid. In sputum smear-negative HIV-infected adults presenting symptoms, its yield is superior to the induced sputum (83). The system must stay in place at least one hour, and probably more in paucibacillary forms (84). A study in Peru showed good tolerability and good acceptability among children aged 4 years and older (85). In a study in Uganda, the tuberculosis detection yield was comparable between string test and induced sputum (86)

MTB reaching the oropharynx and swallowed can ultimately be isolated in stools, where it can be detected by standard microbiological methods or molecular biology methods (87-89). The performance of nucleic acid amplification tests on stools has been assessed and demonstrated in adults

(89, 90) and in children (88, 91). There is growing interest and evidence about the use of Xpert MTB/RIF on stool samples (92, 93), showing that its sensitivity is lower than that of respiratory samples. This is potentially due to the presence of PCR inhibitors in stools; stool flotation methods may be helpful to improve its performance (94).

So far, the implementation of appropriate methods to collect samples in children unable to expectorate remains very limited. In 2002, an audit of hospital practices implemented in all 44 public hospitals treating children with tuberculosis in Malawi showed that only 6% of children assessed had expectorated sputum sampled and only 2% gastric aspirates performed and that none had either induced sputum or laryngeal/nasopharyngeal aspirates performed (95). In 651 pediatric HIV sites surveyed in sub-Saharan Africa in 2012, only 6% and 5% had the capacity to perform induced sputum and gastric aspirates, respectively, while 87% had smear microscopy and 54% mycobacterial culture capacities (96). A recent study implemented in Cambodia aiming at evaluating the quality of childhood tuberculosis diagnosis in 24 high burden operational districts showed that, of 40 clinicians interviewed, only 5% had equipment to perform gastric aspiration, when 27.5% had Xpert machines available at their facility (97).

2.3.2 Immunological tests

Immunological tests, including the tuberculin skin test (TST) and interferon-gamma release assays (IGRAs) measure lasting tuberculosis immune responses due to memory T-cells. Neither TST nor IGRAs can distinguish active tuberculosis disease from latent tuberculosis infection (98). Determining whether someone has active disease rather than latent infection depends on the presence of clinical, radiological, or microbiological findings consistent with tuberculosis disease (17).

TST, which has been in use for more than a hundred years, consists of an intra-dermal injection of 0.1 milliliter of purified protein derivative (PPD) tuberculin, a precipitate of nonspecific molecules obtained from filtrates of sterilized, concentrated mycobacterial cultures. The result, measured by the diameter of the local induration, is read 48 to 72 hours later. A person who has been exposed to the tuberculosis bacilli is expected to mount an immune response around mycobacterial proteins contained in the skin. False positive TST can result from NTM infections or BCG vaccination. Sensitivity of the TST for microbiologically confirmed cases tuberculosis cases in children was estimated by a recent meta-analysis to 86% in high-income countries, and 74% in low income countries (99). In HIV infected children, sensitivity falls down to below 20% due to the cellular immunodeficiency (100, 101).

Interferon Gamma Release Assays (IGRAs) are *in vitro* tests measuring the production of interferon gamma (IFN- γ) by the T-lymphocytes stimulated by MTB-specific antigens, ESAT-6 and CFP-10 (+/- TB 7.7), which are present in *M. tuberculosis* and *M. bovis* but are absent from BCG strains and most atypical mycobacteria. Four commercial tests have been developed: the QuantiFERON ®-TB Gold test (QFT-G), the QuantiFERON ®-TB Gold in-tube (QFT-G IT), and QFT Gold Plus (Qiagen), based on an ELISA technique, and the T-SPOT®.TB test (Oxford Immunotech Ltd, UK BioMérieux), using an ELISpot technique (Enzyme Linked ImmunoSpot) to count INF- γ producing T-cells.

IGRAs have been increasingly proposed as replacement tests of the TST for the screening of LTBI in contacts of TB patients (102-105). Their specificity is significantly higher than that of the TST in BCG vaccinated populations; in non-HIV infected adults with tuberculosis, their sensitivity equals or surpasses that of the TST (106, 107).

Most studies conducted in children aimed at detecting LTBI in low prevalence countries (108). Very few studies evaluated the sensitivity of IGRAs for tuberculosis diagnosis in HIV-infected children

(109, 110). In two studies, ELISpot was significantly more sensitive than TST for the diagnosis of confirmed or probable tuberculosis (73% vs. 36% $p=0.005$; 64% vs. 29% $p=0.003$); similar results were also shown in HIV adults (106, 111, 112). Furthermore, positivity rate of ELISpot in HIV infected children may remain unchanged regardless of CD4 levels (110). This is consistent with studies in adults showing that detection of LTBI by IGRAs is fairly well maintained with immunosuppression although ELISpot tends to perform better (113). Other found more indeterminate results in severely immunocompromised individuals (114, 115). In a recent study in 495 smear-negative South African children, T-SPOT.TB did not have added value beyond clinical data and conventional tests for diagnosis of tuberculosis disease. cough longer than 2 weeks, fever longer than 2 weeks, night sweats, malaise, history of household contact and HIV status were the most important predictors of culture-confirmed tuberculosis (116).

Although these tests fail to distinguish between LTBI and tuberculosis disease (98), active tuberculosis tends to associate with higher IFN- γ responses in adults (98, 117) and in HIV infected children (110). Moreover two studies found that the ratio of cumulated responses to CFP10/ESAT6 antigens on CD4 level of HIV infected had a good diagnostic value for tuberculosis disease (112, 118).

2.3.3 Clinical and radiological features

Due to the low rate of microbiological confirmation, in non-endemic areas, the diagnosis of childhood tuberculosis is based on criteria including: i) a contact with a contagious adult tuberculous index case, ii) a positive tuberculin skin test (TST), iii) suggestive radiographic signs (119, 120). Clinical features may also be helpful but they are often scarce if diagnosis is performed early in the disease evolution. The accuracy of this approach is attenuated by the widespread exposure to MTB in endemic areas (121, 122). In addition, the TST is difficult to interpret in areas where BCG vaccine is widely used. As a result, TB diagnosis in children in endemic areas commonly relies on clinical symptoms and interpretation of chest radiography (CXR) (37).

Symptoms and signs of tuberculosis are most often nonspecific in children. Systemic symptoms and signs include fever (sometimes with night sweats), failure to thrive or weight loss (crossing centile lines during the past 3–6 months, or having lost more than 10% of body weight over any time interval) and unusual fatigue or lethargy(17). Well-characterized symptoms have a better specificity. The interest of a diagnostic approach based on such symptoms has been demonstrated in a prospective study showing that the combined presence of 3 symptoms at inclusion, including unremitting persistent cough for more than 2 weeks, objective weight loss in the last 3 months, fatigue reported by the family constituted a good diagnostic approach of tuberculosis in South African non-HIV infected children (sensitivity 82%, specificity 90%). On the other hand, this symptomatic approach performed poorly in children younger than 3 years, and worse in children infected by HIV (sensitivity 56%, specificity 62%) (100). While typical respiratory symptoms include a persistent, non-remitting cough of more than 2 weeks duration, young children may present more acute cough or wheeze (17, 123). In young children, wheezing unresponsive to bronchodilators may occur from airway compression by enlarged intrathoracic lymph nodes. Physical signs are most often minimal with intra-thoracic tuberculosis. Children rarely present with acute respiratory distress or auscultation anomalies dues to lobar consolidation. Matted lymph node masses in cervical or supraclavicular regions are suggestive of peripheral TB adenitis, a common extrapulmonary location of tuberculosis in children (8, 17).

In HIV-infected children tuberculosis symptoms are most often similar. In Asian HIV-infected children in the TREATAsia Pediatric HIV Observational Database (TApHOD) with approximately half of pulmonary tuberculosis episodes (55.2%) had classical clinical manifestations of PTB. Similarly, Walters et al. showed that most of their HIV/TB co-infected children presented with weight

loss or failure to thrive (66%), persistent cough for >2 weeks (37%), and intermittent fever for >1 week (31%) (28). However, these clinical manifestations were less frequently observed in HIV-infected children than in HIV-uninfected children with definite pulmonary tuberculosis (124), possibly due to the relative inability to develop a full inflammatory reaction to the infection because of HIV-related immunodeficiency.

Chest radiography plays an important role and is largely used for the diagnosis of childhood tuberculosis but faces many practical challenges including low quality of images and insufficient reading skills from frontline clinicians (125). In children below the age of 5, a lateral view can be requested to distinguish thymic images from mediastinal nodes. The spectrum of radiological abnormalities is very broad and signs can be non-specific (17). Certain radiological patterns are however highly suggestive of tuberculosis, such as cavitation, presence of perihilar lymph nodes with airway compression (126, 127), especially when accompanied by recent tuberculosis exposure or proof of MTB infection. In HIV-infected children, specificity of radiographic features is further reduced. Indeed, HIV-infected children present a wide range of possible co-morbidities and co-infections which may affect CXR with similar features to those of tuberculosis (128). In 2001, Norton et al. (129) found a frequency of chronic and transient lung changes associated with various etiologies on CXR of 40.3% and 31.8% respectively in a cohort of 201 HIV-infected infants and children, chronic changes were associated with lower CD4 cell counts.

Differential diagnosis with other pulmonary infections in HIV-infected children.

In HIV-infected children, differential diagnosis of tuberculosis with other pulmonary infections is particularly a problem. In a necropsy study performed in Zambia, the 4 most frequent findings in 180 HIV-infected children dying from respiratory illnesses were pyogenic pneumonia (41%), Pneumocystis jirovecii pneumonia (29%), Cytomegalovirus (CMV) (22%), and tuberculosis (18%) (130). Bacterial pneumonia is very common in all age groups. Pneumocystis jirovecii pneumonia and cytomegalovirus (CMV) are especially common in infants, and lymphoid interstitial pneumonitis (LIP) is common in older children (131, 132). In 72 HIV-infected children assessed for a diagnosis of tuberculosis in Malawi, the final diagnosis was confirmed and probable pulmonary tuberculosis in 5 and 21 children, respectively, and differential diagnoses were LIP in 10, Kaposi sarcoma in 3, bronchiectasis in 3, and unknown in 30 (133). Of 28 HIV-infected children with chronic lung disease in Durban who underwent invasive investigations 16 (57%) had LIP, eight (29%) had tuberculosis and four (14%) had non-specific interstitial pneumonitis (134). At last bronchiectasis is also a cause of chronic respiratory symptoms and chest-radiograph anomalies which can mimic tuberculosis (135).

2.3.4 Scoring systems and diagnostic algorithms for childhood tuberculosis

To overcome difficulties in diagnosis of childhood tuberculosis, different types of diagnostic approaches have been developed to standardize and rationalize tuberculosis diagnosis: point scoring systems, diagnostic classifications, diagnostic algorithms, or combinations of the aforementioned 3 types (136). Point scoring systems or scores are defined by the fact that numerical values or weight are given to each characteristics used. Diagnostic classifications enable to stratify children in into categories such as probable, possible, unlikely tuberculosis. Diagnostic algorithms have a stepwise approach to diagnosis.

Three main point scoring systems have been used widely and adapted: the Kenneth Jones/Stegen Toledo system, published in 1969 in Chile (137), largely used worldwide and still in use in South America notably, the Keith Edwards criteria, originally published in 1987 in Papua New Guinea (138), which have been widely used and adapted and recommended by the WHO (139), and finally, the

Brazil Ministry of Health System which is still used in Brazil (140). Main characteristics of these scores are presented in table 1. Additionally, 3 scoring systems have been developed in separate studies and have not given rise to further publications (141-143). The scoring system mentioned by Tidjani et al. in a BCG study in Benin may have been recommended by WHO at the time of the publication (1986) for case reviews rather than for diagnostic purposes as it includes results of culture and treatment response (143). All these systems have been developed based on expert opinion, characteristics being weighted subjectively. Interestingly, Fourie et al. used a slightly different process, listing clinical characteristics from 794 children with tuberculosis from different settings including European countries, 67 tuberculosis suspects and 18 not-tuberculosis (141). Logarithmic least square methods were used to compile experts' opinions about the contribution of individual characteristics to the diagnosis. None of these systems were therefore developed using recommended methods for diagnostic prediction models and scores.

Diagnostic classifications include an approach developed in Ethiopia (144), a WHO recommended classification that was largely used and evaluated (145, 146), notably in South Africa, an approach used in the Lusaka University Teaching Hospital (147), and a diagnostic approach developed recently in South Africa by Marais et al. and incorporated in latter South African guidelines (100, 148) (Table 1). Of these five approaches, 4 were based on expert opinion, as scoring systems. Only Marais' approach was developed using multivariate tree regression analysis with a 3 class reference standard based on microbiology, chest radiograph, clinical aspects, and treatment response (100). Scores integrating treatment response and/or culture as diagnostic criteria are not fully suited for treatment decision and can be either used in diagnostic confirmation or as reference standard rather than pure baseline diagnostic approaches. Overall, reviews of existing scoring systems and diagnostic classification revealed their limited coherence, frequent absence of a standard definition of symptoms, poor performance in HIV-infected children, and inadequate validation (149, 150). Several studies attempted at validating these scores and diagnostic approaches by evaluating their diagnostic accuracy for tuberculosis defined by a reference standards. Characteristics of these studies and results in terms of sensitivity and specificity are presented in table 2.

Most scoring systems and diagnostic classification have been developed prior to the HIV epidemic and a few studies only validated use of scores in HIV-infected children. The Keith Edward score was recommended with minor modifications for use in HIV-infected children with tuberculosis in the first version of a WHO edited TB/HIV Manual published in 1996 but not in its second edition published in 2004 (139, 151). Van Rheenens showed that the Keith Edwards criteria had a sensitivity of 88% but a very limited specificity of 25% in a prospective study which enrolled 30% of HIV-infected children in Zambia (152), when other prospective studies in non-HIV infected children showed much better combined sensitivity (84 and 91%) and specificity (97 and 88%) (153, 154). Edwards et al. showed that correlation was poor among clinical scoring systems, apart from those related in their development, such as derivate or adaptations of the Stegen Toledo criteria (155). Sensitivities of the various scoring systems did not vary between HIV-infected and HIV uninfected children. The sample size was small however and specificity could not be calculated as there were no children without tuberculosis enrolled in the study. The Marais study which developed the symptomatic approach in South African children included both HIV-infected children and malnourished children and showed that the 3 symptom approach based on unremitting persistent cough for more than 2 weeks, objective weight loss in the last 3 months, fatigue reported by the family, had a much poorer diagnostic performance in malnourished children and in those infected by HIV, in whom sensitivity was 56% and specificity was 62%, as compared to a sensitivity of 82% and a specificity of 90% in non-HIV infected children aged ≥ 3 years (100). Recently, a retrospective study implemented in Brazil evaluated several scoring systems in HIV-infected children assessed for diagnostic of tuberculosis (156): the Tidjani (143), Marais (100), Brazil MOH (157), and Kenneth Jones (137) systems. The study showed poor agreement and that the Tidjani system, used previously in Benin to evaluate BCG vaccination

effectiveness in a global research program initiated by the WHO had a very high specificity (93%) and a sensitivity of 83% when microbiological data was included, and which was only 45% when microbiology was not considered, which makes it less suitable for contexts where it might be lacking and for children without bacteriologic confirmation.

Despite their interest, and due to their heterogeneity, poor performance in HIV infected or malnourished children, scoring systems and diagnostic classifications which were recommended by the WHO (139) are not currently recommended in childhood tuberculosis guidance (7, 8, 158).

Table 1. Scoring systems and diagnostic classifications for tuberculosis diagnosis in children

Approach	Year	Origin	Elaboration	Type	Source data	Adapted by
Kenneth Jones criteria (Stegen-Jones) (137)	1969	Chile	Expert opinion	Point scoring system	Clinical, CXR, bacteriological (AFB), TST	Montenegro (159) (Peru)
Keith Edwards (138)	1987	Papua New Guinea	Expert opinion + numerical adjustment with WHO score	Point scoring system and algorithm	Clinical, TST	WHO–Harries (139)
Brazil Ministry of Health (157)	2002	Brazil	Expert opinion, Kenneth Jones (137), Tidjani (143)	Point scoring system	Clinical, CXR, TST	
Tidjani (143)	1986	Togo International	Expert opinion WHO	Point scoring system	Clinical, CXR, bacteriology (culture), Tx response	
Bergman (142)	1995	Zimbabwe	Symptoms from cohort Subjective weighting	Point scoring system	Bacteriology (culture), clinical, CXR, biological	
Fourie (141)	1998	International	Multiple expert opinion and weighting obtained by mathematical methods	Point scoring system	Clinical, TST	
Graham(160)	2010	International	Expert opinion	Diagnostic algorithm	Clinical, CXR, bacteriology	
Ghidey and Habte (144)	1983	Ethiopia	Expert opinion	Diagnostic classification	Clinical, CXR, TST, bacteriology/histology	Migliori (161) (Uganda)
WHO (145)	1983		Expert opinion	Diagnostic classification	Clinical, CXR, TST, bacteriology (AFB)	Cundall (146) (Kenya) SATVI vaccine trial algorithm (162) Stoltz–Donald (163) (RZA) Kiwanuka (133)(Malawi)
Osborne (147) UTH Lusaka	1995	Zambia	Expert opinion; adapted from Cundall (146) and WHO (145)	Diagnostic classification	Clinical, CXR, bacteriology	Jeena (164) South Africa
Marais (100)	2006	South Africa	Multivariate tree regression analysis; composite reference standard	Diagnostic classification	Clinical	

AFB: acid-fast bacilli; CXR: chest radiograph; TST: tuberculin skin test; Tx treatment.

Table 2. Results of studies evaluating diagnostic accuracy of scoring systems and diagnostic classifications

Scoring system	Study	Year	Country	Sample size	HIV+ % (prevalence)	Reference standard	Diagnostic accuracy/ Validation
Kenneth Jones	Mathur (165)	1974	India	100	NA (pre-HIV)	Clinical diagnosis	Se 73% Se 95% modified criteria
Kenneth Jones	Mehnaz and Arif (166)	2005	Pakistan	100	NA (low)	Clinical control and response to treatment	Se 56% Sp 100% (7 points cut-off) Se 96% Sp 100% (5 points cut-off)
Kenneth Jones	David (156)	2017	Brazil	131	100%	Expert panel review	Se 67.2 Sp 84.2 with microbiology Se 28.1 Sp 84.2 without microbiology
Stegen Toledo	Viani (167)	2008	Mexico	73	100%	Clinical diagnosis	Se 76.9% (7 points cut-off)
Keith Edwards	Van Beekhuizen (168)	1998	Papua New Guinea	301	NA (low)	CXR findings and improvement under Tx	Se 62% Sp 89%
Keith Edwards (WHO modification)	Weismuller (169)	2002	Malawi	150	NA (high)	Clinical diagnosis	Se 61% for all TB Se 54% for PTB; Se 73% for EPTB
Keith Edwards	Van Rheenen (152)	2002	Zambia	147	31.3%	Diagnostic algorithm	Se 88%, Sp 25%, PPV 55%, NPV 67%
Keith Edwards	Narayan (154)	2003	India	65	NA (?)	Clinical diagnosis	Se 91%, Sp 88%
Brazil MOH	Sant'Anna (170)	2004	Brazil	164	NA (?)	Clinical diagnosis & CXR, TST	Se 81.7% (35 points cut-off)
Brazil MOH	Sant'Anna (140)	2006	Brazil	141	NA (?)	Culture and evolution on treatment	Se 58% Sp 98% (40 points cut-offs) Se 89% Sp 86% (30 points cut-offs)
Brazil MOH	David (156)	2017	Brazil	131	100%	Expert panel review	Se 85.0 Sp 39.2 (30 points cut-off) Se 43.8 Sp 76.5 (40 points cut-off)
Tidjani	David (156)	2017	Brazil	131	100%	Expert panel review	Se 82.8 Sp 93.0 with microbiology Se 45.3 Sp 93.0 without microbiology
Marais	Marais (100)	2006	South Africa	428	12.4%	Clinical and CXR criteria	Se 82.3 Sp 90.2 HIV neg > 2y Se 56.2 Sp 61.8 HIV infected

EPTB: extra-pulmonary tuberculosis; MOH: Ministry of health; NA: not available; NPV: negative predictive value; PPV: positive predictive value; Se: sensitivity; Sp: specificity; TB: tuberculosis; Tx: treatment.

2.3.5 Novel biomarkers and technologies

A number of other biomarkers and technologies are currently being developed, tested, validated, or at later stage in the endorsement and country dissemination in the tuberculosis diagnostics “pipeline” (171). This includes a number of sputum based molecular assays developed as alternatives to the GeneXpert system / Xpert test: the Indian TrueNAT micro RT-PCR device, the GeneDrive PCR system, the TB LAMP. The TrueNAT micro RT-PCR device is an IS6110 PCR run on a battery operated device which could probably enable tuberculosis testing at lower levels of care, but requires significantly more handling than the Xpert MTB/RIF. Its performance is close to the Xpert MTB/RIF (172). The GeneDrive is a miniaturized molecular platform which has shown poor performance compared to Xpert MTB/RIF (173). The TB-LAMP is a manual NAAT using loop-mediated isothermal amplification for MTB detection. It has shown good performance compared to Xpert and is now recommended by the WHO as a replacement test for smear microscopy(174). It is currently being tested at large scale in Cameroon.

The ideal true point-of-care test for tuberculosis would be non-sputum based to enable its use for screening of children and patients with suspicions of extra-pulmonary tuberculosis. The urine-based LAM test was developed in such intent and has shown disappointing results (68, 71). There are currently very few non-urine based tests being developed, which further reinforces the need to improve specimen collection in children, as sputum based tests are being improved.

At last, a genomewide analysis of RNA expression in host blood showed that a 51-transcript signature had a sensitivity of 82.9% and a specificity of 83.6% for the diagnosis of culture-confirmed tuberculosis and may have an interest to distinguish tuberculosis from other diseases in African children with and those without HIV infection (175), in line with overall interests for “omics” in tuberculosis and other fields of diagnostics(176). This is however very preliminary work, not yet applicable to routine practice.

2.4 Reference standards for diagnostic studies on tuberculosis in children

Mycobacterial culture, the accepted reference standard for tuberculosis diagnostic studies in adults, cannot be considered as a "gold standard" in children, due to its limited sensitivity. It is therefore accepted that there are a number of tuberculosis cases in children which are not culture-proven. Various approaches have been used to define tuberculosis in these cases as a reference standard for diagnostic studies.

A number of approaches have been used in high HIV-prevalence setting. Bacteriology is always considered to confirm tuberculosis either by culture alone (28) or by both culture and presence of acid-fast bacilli on smear microscopy (100). CXR findings have been used to defined tuberculosis as either radiologically certain when 2 independent experts agreed that the CXR indicated certain tuberculosis in the absence of bacteriologic confirmation (100), or radiologically indicative, where cultures were negative or unavailable, but the CXR [or another radiographic investigation such as a computed tomography (CT)-scan] was considered indicative of tuberculosis (28). A third category was used to identify tuberculosis with a lower degree of certainty. It could be considered as probable tuberculosis defined by the presence of suggestive radiologic signs and good clinical response to tuberculosis treatment i.e. complete symptom resolution and weight gain of 10% of body weight at diagnosis, within 3 months of starting tuberculosis treatment (100), or clinically suspected, where the child presented with tuberculosis symptoms, or extrapulmonary organ involvement together with documented contact and/or a positive tuberculin skin test(28). Not tuberculosis could be defined as spontaneous symptom resolution or no response to tuberculosis therapy in the absence of bacteriologic confirmation or radiologic signs suggestive of tuberculosis (100), or as the outcome if an alternative

diagnosis was established and/or where critical review of the data provided insufficient evidence to support a diagnosis of TB (28).

The University of Cape Town research group including H. Zar and M. Nicol has used the same standard over a series of studies (93, 177, 178). Children were classified as either definite tuberculosis when confirmed by mycobacterial culture on respiratory samples (induced sputum), or -not tuberculosis when all tuberculosis were negative and that resolution of symptoms and signs was documented at 3 month follow-up visit in children who did not receive treatment. All other cases were considered possible tuberculosis, which includes children not receiving treatment, who had no documented recovery at follow-up, and all children placed on treatment in the absence of microbiological confirmation.

Van Rheen en has used an algorithmic approach as a diagnostic standard (Figure 1) (152).

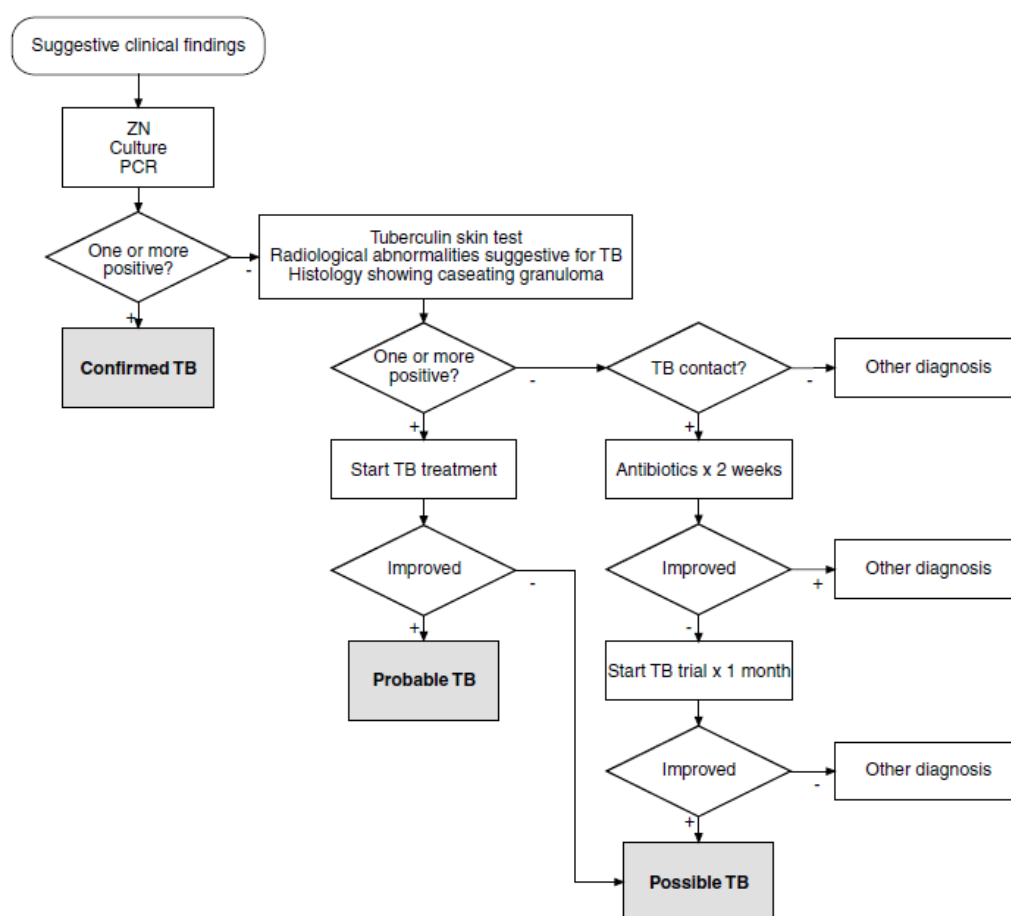


Figure 1. Reference diagnostic algorithm used in a pediatric tuberculosis diagnostic study in Zambia (Van Rheen en (152)).

These different case definitions were quite heterogeneous and did not allow comparing diagnostic performance between studies. To standardize reference case definitions used across studies, a consensus definition was proposed by a group of international experts in 2012: the Clinical Case Definitions for Classification of Intrathoracic Tuberculosis Disease (179). Integrating evolution on treatment and mycobacterial culture results, it classified the children in 5 groups including confirmed tuberculosis, probable tuberculosis, possible tuberculosis, unlikely tuberculosis, and not-tuberculosis. The definition was intended for clinical research use to evaluate diagnostic assays and not for use in

making individual patient diagnoses or treatment decisions such as scoring systems detailed above. The Clinical Case Definition has been updated in 2015 by the same expert group, transforming the 5 group classification into 3 groups including confirmed and unconfirmed tuberculosis and unlikely tuberculosis (65). It now includes Xpert MTB/RIF as a confirmatory bacteriological test and spontaneous symptoms resolution without treatment as a criterion for unlikely tuberculosis. The two versions of the Clinical Case Definition are described in table 3.

Table 3. Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in Children. 2012 and 2015 versions.

	Clinical Case Definition for Classification of Intrathoracic Tuberculosis in Children, 2012 version. (S. Graham et al, JID, 2012)		Updated Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in Children, 2015 version (S. Graham et al, CID, 2015)
Case definition	Refined criteria	Case definition	Refined criteria
Confirmed tuberculosis	1. At least 1 of the signs and symptoms suggestive of tuberculosis AND 2. Microbiological confirmation is obtained	Confirmed tuberculosis	Bacteriological confirmation obtained Requires Mycobacterium tuberculosis to be confirmed (culture or Xpert MTB/RIF assay) from at least 1 respiratory specimen
Probable tuberculosis	1. At least 1 of the signs and symptoms suggestive of tuberculosis AND 2. Chest radiography is consistent with intrathoracic disease due to MTB AND 3. There is at least 1 of the following: (a) A positive clinical response to anti-tuberculosis treatment (b) Documented exposure to M. tuberculosis (c) Immunological evidence of M. tuberculosis infection AND No microbiological confirmation	Unconfirmed tuberculosis	Bacteriological confirmation NOT obtained AND at least 2 of the following: • Symptoms/signs suggestive of tuberculosis (as defined) • Chest radiograph consistent with tuberculosis • Close tuberculosis exposure or immunologic evidence of M. tuberculosis infection • Positive response to tuberculosis treatment (requires documented positive clinical response on tuberculosis treatment—no time duration specified) AND no spontaneous improvement of symptoms in the absence of antituberculosis treatment - With M. tuberculosis infection • Immunological evidence of M. tuberculosis infection (TST and/or IGRA positive) - Without M. tuberculosis infection • No immunological evidence of M. tuberculosis infection
Possible tuberculosis	At least 1 of the signs and symptoms suggestive of tuberculosis AND EITHER (1) One of the following: (a) A positive clinical response to antituberculosis treatment (b) Documented exposure to MTB (c) Immunological evidence of MTB OR (2) Chest radiography is consistent with intrathoracic tuberculosis disease AND No bacteriological confirmation AND Not 1) and 2) present		
Tuberculosis unlikely	Symptomatic but not fitting the above definitions and no alternative diagnosis established	Unlikely tuberculosis	Bacteriological confirmation NOT obtained AND Criteria for “unconfirmed tuberculosis” NOT met (including spontaneous improvement of symptoms in the absence of antituberculosis treatment) - With M. tuberculosis infection • Immunological evidence of M. tuberculosis infection (TST and/or IGRA positive) - Without M. tuberculosis infection • No immunological evidence of MTB infection
Not tuberculosis	Fitting the diagnosis for tuberculosis unlikely but with an established alternative diagnosis.		

In addition to the Clinical Case Definition, the group of international experts issued other recommendations regarding methodological issues in tuberculosis diagnostic studies in children (180). It was notably recommended to use microbiology as a reference standard in early phase proof-of-concept studies despite limited sensitivity of mycobacterial culture, and to report test positivity in the different categories of the proposed Clinical Case Definition.

2.5 Clinical management of tuberculosis-HIV co-infection in children

Clinical management of tuberculosis and HIV-infection is challenging in children with co-infection due to a higher mortality, more severe forms of the disease and poorer prognosis (11, 133, 181, 182). Therapeutic management of tuberculosis/HIV co-infection is complex: heavy treatment burden, pharmacological interactions, double follow-up circuit in sometimes poorly integrated national HIV and tuberculosis vertical programs contribute to significant challenges in management of these children.

Mortality

HIV-infected children with tuberculosis have more severe disease and higher mortality than HIV-negative children (26, 183). Very high mortality rates were reported in co-infected children before the introduction of ART. In a study in 161 children from Côte d'Ivoire, mortality over the duration of the anti-tuberculosis treatment was 3.6 times higher in HIV-infected (57% with CD4<15) than in HIV-negative children, reaching 23% (184). Mortality may however have been underestimated as the study only included non-bacteriologically confirmed pulmonary tuberculosis if children had improved in the first 2 months of treatment. Mortality was similar in 93 South African children of mean age 32 months (54% with CD4 <15%) with 18 (20.7%) deaths occurring during anti-tuberculosis treatment and a total of 34 (39.1%) deaths (30). In Ethiopia, mortality was similar in 58 HIV-infected children followed for one year of whom 22 (41%) died. Kaplan Meier estimates show that 60% of deaths occurred during the first two months of anti-tuberculosis treatment, and that mortality was slightly higher than 25% at treatment completion (185).

Introduction of ART has led to major changes in the dynamics of the epidemic of tuberculosis/HIV co-infection in children. As in adults (186), this has resulted in a reduction in the incidence of tuberculosis in children started on ART (187, 188). Walters et al. reported a fivefold reduction in the number of tuberculosis episodes after ART in South African children and a reduction in the tuberculosis incidence rate from 53.3 per 100 person-years during the 9 months pre-ART initiation to 6.4 per 100 person-years during post-ART follow-up period (28). Similarly, Edmonds et al. showed a twofold reduction in the number of post-ART tuberculosis episodes (32.8% vs. 16.9%) and a reduction of tuberculosis incidence rate from 20.4 per 100 person-years in HIV-infected children who did not receive ART to 10.2 per 100 person-years in children who did in the Democratic Republic of Congo (189). In South-Africa, Tanzania and Western Kenya, tuberculosis incidence was reduced by 70%, 70% and 85% by ART introduction, respectively (53, 190, 191). In the South African study, the incidence rate fell down from 21.1 per 100 person-years to 6.4/100 py (53).

Tuberculosis incidence nevertheless remains higher in children on ART than in the general pediatric population and exposes children to a higher risk of death. In 670 Thai children initiated on ART and followed a median of 7.7 years, tuberculosis incidence was 0.7 per 100 person-years and was associated with a 5-fold increase in risk of death (192). In the ARROW trial, overall incidence of tuberculosis on ART in 1,206 children followed for 3 years was in 1.9/100 child-years, and was highest in the first 12 weeks following ART initiation (8.8/100 child-years versus 1.2/100 child-years

after 52 weeks) (193). The tuberculosis incidence rate was similar in children initiated on ART from 2004 to 2008 in 29 sites in Côte d'Ivoire with 6.3 incident cases /100 child-years during the first 3 months on ART, then dropping at 0.56/100 child-years after 180 days (194).

ART introduction also reduced tuberculosis related mortality in adults (195). A recent meta-analysis showed that of 2792 HIV-infected children hospitalized from 01 January 2007 to 31 January 2015, 10.8% were hospitalized for tuberculosis; 492 (18.8%) died and in-hospital mortality attributable to tuberculosis was 30.1% (95% CI 11.2 to 48.9%) (196).

Tuberculosis treatment

Tuberculosis treatment in children is based on a 4 drug regimen for 6 months as in adults, including rifampin (R), isoniazid (H), ethambutol (E), and pyrazinamide (Z) during the 2 month intensive phase, and RH during the 4 month maintenance phase (8). In HIV-uninfected children, especially in case of lymph node disease or in those without extensive lung disease, a triple therapy combining rifampin, isoniazid, and pyrazinamide be used during the intensive phase. In HIV-infected children, ethambutol should be maintained whatever the disease form and intermittent treatment should be avoided (8, 197). Treatment doses, previously equivalent to adult doses, were revised by the WHO in 2010, based on evidence from 1) pharmacokinetic data in young children showing that higher dosages are required than in adolescents and adults to achieve desired serum concentrations (198); and 2) reports of low toxicity in children with higher dosages (199). Doses were increased for children less than 25 kgs by 100%, 50%, 40%, for isoniazid, rifampin, and pyrazinamide, respectively, which made impractical the use of previous forms of fixed-dose combinations (FDC). New child-friendly FDCs have been developed with currently recommended dosages and have been recently pre-qualified by the WHO.

ART

ART is now recommended in all HIV-infected children regardless of CD4 count, but the optimal timing for its initiation in those receiving anti-tuberculosis treatment is still unclear (200). WHO's recommendation to initiate ART between 2 to 8 weeks after anti-tuberculosis treatment start and earlier when CD4 are below 50 cells/ μ L was based on data from adult trials (8, 201).

Concomitant administration rifampicin - a potent drug metabolizing enzyme inducer - and antiretrovirals is challenging due to drug-drug interactions (202). Efavirenz, one of the Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI), is the recommended first line in case of concomitant anti-tuberculosis treatment due to more important interactions with other NNRTIs (nevirapine) and protease inhibitors. One issue was the contra-indication of efavirenz based ART in children below the age of 3 years and/or 10 kg bodyweight. However data on 45 children treated for tuberculosis with an efavirenz-based regimen were reassuring (203). Rates of virological success are identical between children treated for tuberculosis or not when ART provided is NNRTI, especially efavirenz, around 85 to 90% with HIV RNA below 400 copies/mL (204). However, success rates are lower in children treated with protease inhibitors, most often lopinavir/ritonavir (204). This problem can be overcome with super-boosting of lopinavir with equivalent doses of ritonavir (205). This is however challenging and impractical.

Immune Reconstitution Inflammatory Syndrome (IRIS)

The immune reconstitution inflammatory syndrome (IRIS) is a well-known complication of antiretroviral therapy. It consists of clinical deterioration with marked inflammatory signs in the context of a rapid restoration of pathogen specific immune responses to opportunistic infections (206-208). There are two types of IRIS: 'paradoxical IRIS', which is due to the worsening of a previously

treated infection and ‘unmasking IRIS’, which is the presentation of a new infection. Although paradoxical tuberculosis-associated IRIS has been widely studied and reported in adults (206, 207, 209), little has been described in children (210-214). Data on incidence of IRIS in children, including unmasking IRIS and paradoxical IRIS, are scarce and mostly based on retrospective studies; incidence seems to range from 19% to 38 % (212-218). Identified risk factors of IRIS in children are low CD4 cell count, low CD8 cell count, malnutrition, high pretreatment HIV viral load, male sex (212-216). Although several pathogens have been reported, IRIS linked to mycobacteria is the most frequent in children. It involves Bacille Calmette Guérin IRIS in young children, NTM in older children, or MTB in endemic countries (212-214, 219-222). Unmasking tuberculosis IRIS was the more frequent type of IRIS reported in children who started ART in pediatric cohorts in high burden countries. A review from South Africa has shown an incidence of paradoxical tuberculosis-associated IRIS of 7.4% (211). This may be due to the late introduction of ART in children treated for tuberculosis in some cases, or low detection of tuberculosis in children pre-ART, leading mostly to unmasking tuberculosis IRIS, or eventually to early ART mortality. It has indeed been showed that although mortality and AIDS related morbidity decreases with introduction of ART in children (223), mortality remains high in the first months of ART (210, 224). The potential role of IRIS in this early mortality is not well understood (225). In adults, delay between anti-tuberculosis treatment and introduction of ART is a powerful risk factor of developing IRIS, and improvement of tuberculosis screening and diagnosis significantly reduces the incidence of tuberculosis unmasking IRIS (226-228). Introducing ART early will probably further reduce mortality in HIV-infected children; however, the price to pay should be an increase in IRIS incidence (229-231).

Isoniazid Preventive Therapy

The WHO currently recommends initiating on Isoniazid Preventive Therapy for a duration of 6 months all HIV-infected children that enter care and who do not present fever, cough, weight loss, or history of contact with a tuberculosis case, and to consider it in symptomatic children if tuberculosis has been ruled out (40). IPT has also been recommended by WHO for many years in children below the age of 5 years who are in close contact with a source case of smear-positive pulmonary tuberculosis, as they are at high risk of tuberculosis infection and subsequent development of tuberculosis disease. However there are major programmatic gaps in the implementation of this recommendation (232). Data in HIV-infected adults show the efficacy of IPT to reduce tuberculosis incidence and mortality (233, 234). In children a meta-analysis has shown that IPT reduces the risk of developing tuberculosis by 59% among children aged 15 years or younger, excluding children initiated during early infancy for primary prophylaxis. However, authors estimated that further studies are needed to assess effects on mortality and to determine prophylaxis effectiveness in very young children and among HIV-infected children (235).

2.6 The ANRS 12229 PAANTHER 01 Study

All research and publications in this thesis have been done in the framework of the ANRS 12229 PAANTHER (Pediatric Asian African Network for Tuberculosis and HIV Research) 01 study. The study was implemented in sites from the PAANTHER network (Pediatric Asian African Network for Tuberculosis and HIV Research), which was established during the ANRS 12194 project and reinforced for the ANRS 12229 PAANTHER 01 study. As pediatric HIV sites, most were confronted to the same clinical questions and need to work on the issue of improving diagnosis of tuberculosis in HIV-infected children. There were 2 participating countries from South-East Asia, Cambodia and

Vietnam, with estimated tuberculosis incidence rates of 437 and 199 cases per 100,000 population in adults, and 2 countries from Africa, Burkina Faso and Cameroon, with incidence rates of 55 and 177 cases per 100,000 population. Cambodia and Vietnam were part of the WHO list of the 22 high-burden countries.

2.6.1 Study design

The ANRS 12229 PAANTHER 01 study was a diagnostic study based on an international (Burkina Faso, Cambodia, Cameroon, Vietnam), multicentric and prospective cohort of HIV-infected children presenting a suspicion of tuberculosis. After initial clinical, radiographic, and bacteriological evaluation, a tuberculosis diagnosis was done by the attending clinician, according to national recommendations. Every child was followed up for 6 month, regardless of the diagnosis. For study purposes, additional diagnostic methods were added and evaluated, including IGRAs, alternative specimen collection methods (nasopharyngeal aspirates, stool samples, and string test), and improved laboratory techniques (Auramine O stains and LED microscopy replacing Ziehl-Neelsen stains and standard microscopy). The Xpert MTB/RIF test was performed prospectively on 5 to 6 samples per child: 2 to 3 standard samples (expectorated sputum or gastric aspirates) and 3 alternative samples. The ANRS 12229 PAANTHER 01 enrolled 438 children.

The study was approved by national ethics committees of the 4 participating countries and relevant institutional review boards and national authorities. The ANRS 12229 PAANTHER 01 study is registered at ClinicalTrials.gov (identifier NCT01331811).

2.6.2 Study population

Children with the following criteria were eligible for the study.

- **Inclusion criteria**

- Children aged from 0 to 13 years
- HIV-1 Infection, as documented at any time prior to study entry, according to the national testing procedures and the age of the child at the time of testing
- On Highly Active AntiRetroviral Therapy (ART) or not
- Suspicion of tuberculosis based on one or more of the following signs:
 - Persistent cough for more than 2 weeks
 - Persistent fever for more than 2 weeks
 - Recent failure to thrive (documented clear deviation from a previous growth trajectory in the last 3 months or z score weight / age < 2)
 - Failure of broad spectrum antibiotherapy for a pulmonary infection
 - Suggestive thoracic radiographic anomaly
- Informed consent signed before any trial procedure by at least one parent or guardian

- **Non-inclusion criteria**

- History of any anti-tuberculosis treatment started in the past 2 years before enrollment

2.6.3 Study settings

Clinical sites participating to the study were the following:

- Pediatric Department, University Teaching Hospital (CHU) Sourô Sanou, Bobo Dioulasso, Burkina Faso
- National Pediatric Hospital, Phnom-Penh, Cambodia
- Angkor Hospital for Children, Siem Reap, Cambodia
- Centre Mère Enfant de la Fondation Chantal Biya, Yaounde, Cameroon
- Hôpital de la Caisse d'Essos, Yaounde, Cameroon
- Pham Ngoc Thach Hospital, Ho Chi Minh City, Vietnam
- Pediatric Hospital Nhi Dong 1, Ho Chi Minh City, Vietnam
- Pediatric Hospital Nhi Dong 2, Ho Chi Minh City, Vietnam

All sites were involved in follow-up of cohorts of HIV-infected children, at the exception of the Pham Ngoc Thach Hospital in Vietnam, which is a respiratory diseases hospital and the reference center for tuberculosis.

Regional and national project coordination, as well as data management and monitoring were done by the Epidemiology and Public Health Unit of the Institut Pasteur in Cambodia, the ANRS site in Cameroon, and the Centre Pasteur du Cameroon. Reference laboratories for the project were the Sourou Sanou UTH laboratory and the Centre Muraz in Bobo Dioulasso, Burkina Faso, the Institut Pasteur laboratory in Phnom Penh, Cambodia, the Centre Pasteur du Cameroun laboratory in Yaounde, Cameroon, and the Pham Ngoc Thach Hospital and the Institut Pasteur laboratory in Ho Chi Minh City, Vietnam.

2.6.4 Study procedures

- **Informed consent and inclusion visit**

After signed informed consent by the parent or guardian, all eligible children could be enrolled in the study. The inclusion visit included an interview of parent/guardian and possibly of the child on medical history and history of treatment and ongoing treatment, history of the disease and symptoms at home, including features of cough if present, and a complete clinical examination. A blood sample was drawn for Complete Blood Count (CBC), transaminases, alkaline phosphatase, lactate dehydrogenase, total and conjugated bilirubin in case of jaundice, uric acid, CD4 T-cell count (absolute count and percentage), HIV-1 viral load, and the Quantiferon Gold In-Tube test. In addition, children in Cambodia had a T-Spot test performed. A chest radiograph (anteroposterior and left lateral views for children aged ≤ 5 years, and postero-anterior views for children older than 5 years) as well as an abdominal ultrasound were performed. A TST was performed and results were read after 48-72 hours using the ballpoint pen technique (considered positive if ≥ 5 mm in HIV-infected children).

- **Bacteriological specimen collection and tests**

Collection of initial bacteriological specimens was planned over a period of 3 days. Gastric aspirates, sputum samples and string test were performed according to the child's age, as shown in the following table.

Age	Day 1	Day 2	Day 3
All	Nasopharyngeal aspirates		
< 4 years	Gastric aspirate	Gastric aspirate	Gastric aspirate
4 to 9 years			String test
≥ 10 years	Sputum (2)	Sputum	

Gastric aspirates were performed early morning after overnight fasting, in supine position, before any rise in case of hospitalization and after 1 hour of supine for children seen in consultation. Nasopharyngeal aspirate were performed collecting the contents of the oropharynx by mechanical suction through a graduated suction tube inserted into the nostril while the child was seated, which provoked a cough reflex. String test was performed using the Pediatric Entero-test® (HDC Corporation, Milpitas, California, USA). It is a device consisting of a cotton string with a length of 90 cm coiled into a gelatin capsule. The string unravels as the capsule is swallowed by the child, fasting, in the morning and the upper extremity of the string is attached to the cheek of the child by a plaster. The device was left in place for 2 hours during which the child could be engaged in quiet activities and games. The string was removed after 2 hours by a nurse with a gentle pull of 5 seconds and was immediately deposited into 3 ml of 0.9% saline serum, secondarily agitated by a vortex agitator in the site laboratory.

A direct exam for detection of Acid-Fast Bacilli was done by Auramine staining and fluorescence microscopy after decontamination and centrifugation for all samples, excepting stools which were stained by Ziehl-Neelsen method and examined using standard microscopy. Mycobacterial culture was be done in liquid medium (MGIT) by an automated method (BACTEC 960) or on solid media (Lowenstein Jensen). Identification of mycobacteria was done by the molecular method Gen-Probe®, immunochromatographic MPT64 antigen tests, or classic biochemical methods. Drug sensibility testing (DST) was performed for 1st line TB drugs.

Xpert MTB/RIF tests were performed on untreated respiratory samples and stool samples or on decontaminated pellet in case of low quantity of sample. The tests were performed and interpreted according to the manufacturer's instruction.

- **Clinician's diagnosis and initiation of ART and anti-tuberculosis treatment**

After clinical, biological and radiological investigations, the clinician had to make a diagnosis of tuberculosis, or not, according to the national tuberculosis program criteria, without access to the result of IGRAs. Results of the Xpert MTB/RIF were sent back to the sites clinicians by the coordination center once on-site investigators had performed all tests and assessed the child for the diagnosis of tuberculosis with access to smear microscopy results (Auramine and ZN staining), to assess the diagnostic utility of this test.

In case of tuberculosis diagnosis, the anti-tuberculosis treatment was started as recommended by the national protocol, as soon as possible after completion of the bacteriological samples planned per protocol. Antiretroviral Treatment was started within 2 to 8 weeks or modified if needed according to national recommendations. Clinical evolution on treatment is one of the criteria for validation of reference standard. Adherence to the anti-tuberculosis treatment and ART was evaluated by pill count and by an adherence questionnaire administered to parent/guardian and child.

- **Follow up visits**

Four follow-up visits were planned for all children, regardless of the diagnosis, at month (M) 1, M2, M3, and M6. Any change in ART or tuberculosis treatment was collected. In case of suspicion of IRIS, complementary examinations were performed. A final evaluation was done at M6. In addition to the full clinical evaluation, a biological and radiographic evaluation of tuberculosis, drug toxicity, and HIV-infection (CD4 count and plasma viral load) was performed.

- **Case review and reference diagnosis**

The reference diagnosis was based on retrospective case review and use of the Intrathoracic Tuberculosis Definitions for Diagnostic Research in Children (236).the updated version of the case definition was used (65).

2.7 Personal involvement in the study

This thesis is the achievement of 10 years of personal work on tuberculosis in HIV-infected children. Working from 2005 to 2007 as the medical coordinator for the French Red Cross program in Phnom Penh, Cambodia, I provided support to the HIV pediatric outpatient center of the National Pediatric Hospital and to the HIV and tuberculosis inpatient department, both led by Dr. Ung Vibol. Confronted, both as a clinician and program manager, to the challenges in diagnosing tuberculosis in HIV-infected children, I presented an abstract on these challenges, on behalf of my colleagues, at the 4th Francophone HIV conference in Paris on 31st March 2007¹ (237). Concomitant meetings in Paris with colleagues from African ANRS research sites and HIV pediatricians from Necker hospital confronted to the same challenges and scientific questions led to the development of a collaborative network that would implement the PAANTHER 01 study.

In March 2008, we submitted on behalf of this group, as co-principal investigators with Pr. Stéphane Blanche, from the Necker Hospital, Paris, a request to the ANRS for an initiation contract, the ANRS 12194 project, entitled “Diagnosis of Tuberculosis in HIV-infected children. Feasibility study of a multicentric (Africa - Asia) research in Burkina Faso, Cambodia, Cameroon, and Vietnam: setting of clinicians network, determination of diagnostic methods, evaluation of recruitment, and quality of laboratories”. Our objectives were: 1) to evaluate the feasibility of a multicentric research project on improvement of tuberculosis diagnosis in HIV infected children in various sites, especially on the microbiological aspects, 2) to finalize the protocol on tuberculosis in HIV-infected children to submit to the next following ANRS grant application session (September 15th, 2008), 3) to set up a pediatric North-South (African- Asian) network of clinicians, biologists and epidemiologists in order to develop multicentric research projects on tuberculosis -HIV co-infection in children. We finally submitted the PAANTHER 01 study protocol to the ANRS in September 2009. The ANRS 12229 PAANTHER 01 study was approved by the ANRS early 2010 for an implementation restricted to Asia. The extension to African sites and inclusion of Xpert MTB/RIF was later obtained, with the support of Dr. Philippe Msellati, and Drs François-Xavier Blanc and Didier Laureillard, respectively.

As the PAANTHER 01 coordinating investigator with co-investigators Dr. Sam Sophan, and later Dr. Ung Vibol, I actively participated to all steps of the study life: protocol and procedures writing, case report form (CRF) design, training of clinicians in Asia, writing of the statistical analysis plan, overall operational coordination, in collaboration with Drs. Laurence Borand and Philippe Msellati, regional coordinators for Asia and Africa, and scientific coordination. The study inclusions lasted from April 2011, date of research start in Cambodia, to May 2014 and follow-up ended in December 2014.

As I had moved on from my position as medical coordinator with the French Red Cross to be the clinical coordinator on the CAMELIA trial in Cambodia, I became more interested in clinical research and I was challenged by technical questions around clinical research methodology. I finalized a Master in Public Health specialized in clinical epidemiology at the University of Bordeaux in 2012 and subsequently work in the Inserm U897 Epidemiology and Public Health research center. I joined the

¹ [Management of tuberculosis HIV co-infection in children at the National Pediatric Hospital, Phnom Penh, Cambodia]. O. Marcy, S. Sam, V. Ung, B. Chan, T.C. Eap, V. Guilletmet, T. Ban, T. Tran Minh, C. Courpotin, C.Y. Meng. 4th Francophone Conference on HIV/AIDS in Paris, France, 29-31 March 2007

Infectious Disease in Low Income Countries in 2014 where I was able to work on my thesis while being the international trial manager in charge of the ANRS 12300 Reflate TB 2 trial testing raltegravir based ART as an alternative to efavirenz for ART in adults co-infected with HIV and tuberculosis.

Following 6 months of intensive data management until database freeze later on in 2015, the first publication of the PAANTHER 01 study was published in the Clinical Infectious Diseases journal. Statistics were performed by a colleague in charge of the data management. I performed all other statistical analyses for publications presented in this thesis.

2.8 Thesis justification and objectives

Tuberculosis diagnostic tests available in developing countries, as well as the diagnostic approaches recommended by the national tuberculosis programs and the WHO, need better sensitivity and specificity, especially in children infected with HIV.

Several interesting options could improve the diagnosis of tuberculosis in HIV-infected children and require in-depth evaluation: the use of Xpert MTB / RIF tests, particularly on alternative specimens, the replacement of TST by IGRAs for the immunological diagnosis of tuberculosis infection, the replacement of invasive sample collection methods by alternative methods in ambulatory care such as string tests or nasopharyngeal aspirations, the contribution of routine stool examination and ultrasound, and the possibility of improving the diagnostic approaches currently recommended in countries with high tuberculosis burden.

These objectives are among the research priorities on childhood tuberculosis identified by WHO in 2007 (238). The results of this study could potentially be used to review and improve national guidelines for the diagnosis of tuberculosis in children infected with HIV.

Research on the diagnosis of tuberculosis is confronted with methodological difficulties linked to the absence of a perfect standard gold standard test. A clinical definition of cases for the classification of intra-thoracic tuberculosis has been proposed for research in 2012 by Graham *et al* under the auspices of the US National Institutes of Health and updated since (65, 236). For the time being, the updated definition has not been formally used in recent publications in children and the former one has shown its limitations (239).

Presentation of objectives

The primary objective of the thesis is to develop an algorithm for the diagnosis of tuberculosis in HIV-infected children in developing countries with high tuberculosis burden. As secondary objective, it will also assess the diagnostic value of the Xpert MTB/RIF test for the diagnosis of tuberculosis and its performance on samples collected by alternative methods such as the string test, nasopharyngeal aspirates, and stool samples, the diagnostic value of chest radiography, and it will assess mortality and its determinants in ART-naïve children presenting with suspected tuberculosis at 6 months. Additionally, it will explore the methodological problems raised by the absence of a “gold standard” reference diagnosis and the use of a standardized definition of tuberculosis cases for research in the context of pediatric HIV infection.

3 Mortality and its determinants in ART-naïve HIV-infected children with a suspicion of tuberculosis, an observational cohort study

3.1 Research question and background

Tuberculosis is the leading cause of death in HIV-infected children accounting for 1/3 of all deaths in this group. Recent mathematical modelling work has estimated that 90% of HIV-infected children with tuberculosis die without accessing anti-tuberculosis treatment (5). However, observational studies report high mortality rates in these children, even during the first months of anti-tuberculosis treatment (240). In ART naïve children presenting a suspicion of tuberculosis, clinicians have to make a double decision pertaining to anti-tuberculosis treatment and ART initiation.

Understanding better which children are most at risk of dying and what are the determinants of mortality, including treatment choices is of major importance for clinicians.

3.2 Marcy *et al* – Lancet HIV – in press

This study has focused on the 266 ART-naïve children enrolled in the PAANTHER 01 study. Its findings have been presented at the Union Conference in Cape Town, 2015, and at the International AIDS conference and the TB 2016 conferences in Durban, South Africa, in July 2016. It has been accepted for publication by the Lancet HIV.

A supplementary appendix has been submitted to the journal for interested readers and is presented in Appendix 3 of this thesis.

Mortality and its determinants in ART-naive HIV-infected children with a suspicion of tuberculosis, an observational cohort study

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ABSTRACT

Background: Tuberculosis is a major cause of morbi-mortality in HIV-infected children, but is difficult to diagnose. We studied mortality and its determinants in antiretroviral treatment (ART)-naïve HIV-infected children presenting with a suspicion of tuberculosis.

Methods: HIV-infected children aged ≤ 13 years with a suspicion of tuberculosis were followed 6 months in the ANRS 12229 PAANTHER 01 cohort (Burkina Faso, Cambodia, Cameroon, Vietnam). Children started ART and/or anti-tuberculosis treatment at the clinician's discretion and were retrospectively classified in 3 groups according to tuberculosis documentation (confirmed by culture or Xpert MTB/RIF, unconfirmed, and unlikely). We assessed mortality and associated factors using Kaplan-Meier methods and Cox proportional-hazard models.

Findings: 266 ART-naïve children (confirmed-tuberculosis, $n=40$; unconfirmed-tuberculosis, $n=119$; unlikely-tuberculosis, $n=107$), were followed for 112.5 person-years. 154 (57.9%) started anti-tuberculosis treatment, and 212 (80.9%) started ART; 50 (19.5%) children died. Mortality was significantly higher in children with confirmed-tuberculosis (14 deaths) compared to unconfirmed-tuberculosis (19 deaths) and unlikely-tuberculosis (17 deaths) (log rank=0.014), and lower in children with confirmed or unconfirmed tuberculosis who started anti-tuberculosis treatment ($p<0.0001$ and <0.0001). In multivariate analysis, ART started during the first month of follow-up [hazard ratio (HR) 0.084; 95%CI 0.010-0.670], confirmed-tuberculosis, young age, CD4<10%, miliary features, and elevated serum transaminases were all independently associated with mortality.

Interpretation: Mortality was high in the first 6 months following suspicion of tuberculosis in ART-naïve children. ART should be introduced early, particularly in children with factors associated to high mortality. Documented or empirical anti-tuberculosis treatment decision should be accelerated to reduce mortality and allow early ART introduction.

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INTRODUCTION

Tuberculosis remains a major cause of morbidity and mortality in HIV-infected children.¹ In 2015, the World Health Organization (WHO) estimated the annual burden of childhood tuberculosis to 1 million new cases and 210,000 deaths, including 41,000 deaths in HIV-infected children.² There were no incidence estimates in HIV-infected children but mechanistic modelling which contributed to the WHO report estimated HIV-prevalence to 5.0% (IQR 2.4–10.1) of childhood tuberculosis cases in the WHO-defined high-burden countries, reaching more than 25% in South Africa and Zimbabwe.³

The WHO recommends investigating for tuberculosis all HIV-infected children presenting with poor weight gain, fever, current cough or history of contact with a tuberculosis case.⁴ Clinical management of HIV-infected children with a suspicion of tuberculosis is challenging, especially in resource-limited settings.⁵ Diagnostic confirmation is seldom reached due to the paucibacillary nature of the disease, difficulty to self-expectorate in young children, and therefore low proportion of bacteriologically-confirmed tuberculosis. In addition, extra-pulmonary tuberculosis, which is frequent in HIV-infected children with severe immunodeficiency further complicates diagnosis.¹ Tuberculosis treatment decision is generally based upon clinical features such as fever, asthenia, weight loss, chronic cough and radiological features which show poor specificity in the context of advanced immunosuppression. In resource-constrained settings, chest computerized tomography (CT)-scan and other elaborate tests or technologies are most-often unavailable.

The lack of diagnostic certainty leads to, on the one hand, a large proportion of over-diagnosis and empirical tuberculosis treatment, and on the other hand, under-diagnosis and subsequent mortality.^{6,7} The decision to start antiretroviral treatment (ART) and its timing also has to be considered, in a context where disease forms are more severe and mortality is higher than in HIV-negative children.^{8,9}

In adults, recommendations for symptoms-based screening and use of Xpert MTB/RIF (Xpert), increased availability of the Xpert, and recommendations on appropriate timing for ART initiation contributed to facilitate optimized care and treatment, and reduction in mortality.¹⁰ In HIV-infected children there is much less clarity about the appropriate clinical management in case of suspicion of tuberculosis despite the high related mortality. ART is

now recommended in all HIV-infected children regardless of CD4 count, but the optimal timing for its initiation in those receiving anti-tuberculosis treatment is still unclear.¹¹ WHO's recommendation to initiate ART between 2 to 8 weeks after anti-tuberculosis treatment start and earlier when CD4 are below 50 cells/ μ L was based on data from adult trials.^{12,13}

Identifying children at high risk of mortality among HIV-infected children presenting with a suspicion of tuberculosis is a major step for appropriate treatment decision. We assessed mortality and its determinants including ART and anti-tuberculosis treatment decision, and final tuberculosis diagnosis, in a cohort of ART-naïve HIV-infected children presenting with a suspicion of tuberculosis in four resource-limited countries in Africa and South-East Asia.

METHODS

Study design and patients

The ANRS 12229 PAANTHER 01 study was a diagnostic cohort study conducted in eight hospitals in Burkina Faso, Cambodia, Cameroon, and Vietnam (Appendix, page 2). The inclusion procedures and study design have been described elsewhere.¹⁴ In brief, it enrolled HIV-infected children aged ≤ 13 years with a suspicion of intra-thoracic tuberculosis based on at least one of the following: 1) persistent cough and/or 2) fever > 2 weeks, 3) failure to thrive, defined as recent deviation in the growth curve or a weight for age Z score $< -2SD$, 4) failure of antibiotics for a pulmonary infection, or 5) suggestive chest X-ray (CXR), excluding those with anti-tuberculosis treatment started within 2 years before inclusion. We restricted this analysis to children who were ART-naïve at inclusion.

The study was approved by relevant national ethics committees, institutional review boards, and national authorities (Appendix, page 2). The ANRS 12229 PAANTHER 01 study is registered at ClinicalTrials.gov (identifier NCT01331811).

Procedures and definitions

After parent or guardian informed consent, all children underwent a complete physical exam, a CXR, an abdominal ultrasonography, blood samples for HIV RNA, CD4 cell count and percentage, complete blood count, and transaminases measurements, and specimen collection for Xpert, acid-fast smear microscopy, and mycobacterial culture. ART and anti-tuberculosis treatment were initiated at the discretion of the treating physician. All children were followed in the study for 6 months, with visits at 1, 2, 3, and 6 months (M6) following inclusion.

We retrospectively classified children in three tuberculosis reference diagnosis groups as confirmed, unconfirmed, or unlikely tuberculosis, using the updated version of the Intrathoracic Tuberculosis Definitions for Diagnostic Research in Children (Table S1, appendix, page 3).¹⁵ CXR were reviewed independently by two readers blinded to patient data; discordant opinions were resolved by a third reader.

Children's clinical status was assessed using the Lansky play performance score, WAZ, and retrospectively defined as severe in case of fever $>38^{\circ}\text{C}$, and/or age-defined tachypnea, and/or tachycardia, adapted from danger-signs proposed in HIV-infected adults with a suspicion of tuberculosis.^{16,17} We used the WHO immunological classification to categorize immunodeficiency as either not-significant, mild or moderate, and severe, and considered CD4 below 10%, a level previously associated with high mortality across all ages, as very severe immunodeficiency.^{18,19}

Statistical analysis

Death was the event of interest, and the time axis in the survival analysis was the time elapsed from inclusion in the study. Follow-up was either until death, loss to follow-up, study withdrawal, the final M6 study visit, or 210 days after inclusion, whichever occurred first.

We compared baseline characteristics between groups using Student t-test or Kruskal-Wallis test and Pearson's chi-square or Fisher's exact test as appropriate. We used Kaplan-Meier survival analysis to estimate probability of survival, and used the log-rank test to compare survival across tuberculosis reference diagnosis groups, and impact of anti-tuberculosis treatment. Since failure of anti-tuberculosis treatment contributed to the classification as unlikely tuberculosis, we did not assess the link between mortality and anti-tuberculosis treatment in this group.

We assessed impact of tuberculosis reference diagnosis, and ART, on mortality, as main explanatory variables, and identified additional factors associated with mortality using the Cox proportional-hazard model. We handled ART as a time-dependent variable which was set to zero at each time before ART initiation, and one after ART initiation. We hypothesized that

ART impact on mortality would be higher during the first month of follow-up and thus allowed in the Cox model a different effect of ART during the first month of follow-up and thereafter. Additional factors considered were those previously reported as associated to mortality in children with tuberculosis and/or HIV-infection and those factors that were clinically relevant. In addition to main explanatory variables, we entered in the multivariate model factors associated with mortality in univariate analysis with a p-value <0.20 . We applied a manual backward selection procedure to identify factors independently associated with the outcome, forcing into the model the main explanatory variables. Factors associated with increased baseline aspartate aminotransferase (AST), an unexpected predictor of mortality in ART-naïve children, were assessed by ordinal logistic regression.

A p-value <0.05 was considered significant. All analyses were performed using the SAS software version 9.3 (SAS Institute, Cary, NC, USA).

Role of the funding source

The funders of this study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Of 438 children enrolled in the study from 27 April 2011 to 31 May 2014; 266 were ART-naïve and were included in this analysis. Their median age was 6.1 [interquartile range (IQR): 2.3 – 9.1] years and 137 (51.5%) were male (Table 1). A large proportion were underweight and severely immunocompromised with median weight-for-age Z score (WAZ) and CD4% of -2.5 (IQR: -3.6 to -1.9) and 9.0 (IQR: 2.0 – 18.0), respectively. More than half had increased baseline AST, which was associated with young age, CD4 $<10\%$, high HIV RNA, low hemoglobin, hepatomegaly on abdominal ultrasonography, and the country (Table S2, Appendix page 4). 37 (14.4%) children had smear-positive or Xpert-positive results available at baseline and 180 (73.5%) had CXR features consistent with tuberculosis. Overall, tuberculosis was confirmed by culture or Xpert in 40 (15.0%) children, and 119 (44.7%) were classified as unconfirmed tuberculosis (Table 1). Children were followed up for a median

time of 5.9 (IQR: 5.5-6.2) months with a total of 112.5 person-years of follow-up accrued during the study.

A total of 50 (18.8%) children died during follow-up. Only 5 (1.9%) children were lost to follow-up and 5 (1.9%) withdrew from the study. 27 (54.0%) deaths occurred during the first month of follow-up and 39 (78.0%) during the first 2 months (Figure 1). The probabilities of survival at 2 and 6 months were 85.0% (95%CI 80.7 - 89.4) and 80.7% (95%CI 75.8 – 85.5), respectively.

Mortality was significantly higher in those with confirmed tuberculosis compared to those with unconfirmed and unlikely tuberculosis with 6-month survival probabilities of 65.0% (IC95% 50.2 – 79.8), 83.5% (95%CI 76.8 – 90.3), and 83.5% (95%CI 76.3 – 90.7), respectively (log rank=0.014) (Figure 1). Deaths occurred later in unconfirmed tuberculosis than in confirmed and unlikely tuberculosis, with median times to death of 45 (IQR: 29 – 78), 21 (IQR: 10 – 54), and 15 (IQR: 3 - 29) days, respectively ($p=0.005$). In all 3 diagnostic groups, children who died were younger, and had higher HIV RNA and AST, and a lower proportion started ART (Table 2). In the confirmed tuberculosis group, 5 (41.7%) children who died had miliary features on CXR compared to 1 (3.7%) in those alive at M6 ($p=0.007$). In the unconfirmed tuberculosis group, 12 (63.2%) children who died were classified as severe, compared to 35 (36.1%) in those alive at M6 ($p=0.028$).

During follow-up, 154 (57.9%) children started anti-tuberculosis treatment after a median time of 7 (IQR: 5 – 11) days. Anti-tuberculosis treatment was associated with increased survival in both children with confirmed and unconfirmed tuberculosis (log rank $p<0.0001$ and <0.0001) (Figure 1). Time to anti-tuberculosis treatment did not differ significantly between those who died and those alive at M6 (Table 2). Only 2 children with confirmed tuberculosis were not treated for tuberculosis. Notably, of 12 children with confirmed or unconfirmed tuberculosis who did not receive anti-tuberculosis treatment, 8 died and 2 were lost to follow-up.

Overall, 212 (79.7%) children started ART after a median time of 24 (IQR: 14 – 41) days, including 80 (71.4% of 112) not treated for tuberculosis, and 132 (85.7% of 154) treated for tuberculosis. Children treated for tuberculosis started ART later than the other [30.5 (IQR 17.0 – 41.5) Vs. 14.0 (IQR 11.0 – 32.5) days; $p<0.0001$]. 26 deaths occurred before and 24

after ART initiation. Initiation of ART during follow-up, whatever the timing, was associated with a strong decrease in mortality ($p < 0.00001$).

In the multivariate analysis, ART was associated with an estimated 91.6% reduction in the risk of death over the first month of the study [hazard ratio (HR) 0.084; 95%CI 0.010-0.670], but not afterward (HR 1.133; 95%CI 0.244 – 5.271) (Table 3). Confirmed tuberculosis was associated with a 6-fold increase in the risk of death (HR 6.331; 95%CI 2.150 - 18.642) compared to unconfirmed and unlikely tuberculosis. Additional factors independently associated with increased mortality were young age (< 2 years), CD4 $< 10\%$, miliary radiographic patterns, and elevated AST ($> 2.5 \times \text{ULN}$). In a model considering only those factors that were available at baseline, smear or Xpert-positivity was associated with a 4-fold increase in the risk of death (HR 3.952; IC95% 1.645 – 9.493).

DISCUSSION

Mortality remained extremely high in the first 6 months following suspicion of tuberculosis in ART-naïve HIV-infected children, despite access to anti-tuberculosis treatment and ART. To our knowledge, this is the first study addressing mortality and associated factors in relation with diagnosis and treatment decision in HIV-infected children with a suspicion of tuberculosis. Overall, mortality was similar to that previously observed in a cohort of HIV-infected children with tuberculosis in Malawi, and much higher to that observed in a South African cohort.^{20,21}

Mortality was higher in children with confirmed tuberculosis than in those with unconfirmed and unlikely tuberculosis. The high mortality observed in confirmed tuberculosis despite access to treatment may be due to more severe forms of the disease with higher bacillary loads, as reflected by bacteriological confirmation, or disseminated forms, as reflected by miliary features.^{22,23} Bacteriological confirmation by smear or Xpert, which can be obtained within hours of clinical suspicion, was associated with a 4-fold increase in mortality. In the unconfirmed tuberculosis group, 2/3 of those who died were classified as severe, based on fever, tachycardia or tachypnea. These easy-to-collect vital signs were identified as danger signs associated with mortality and benefit of empirical anti-tuberculosis treatment in HIV-infected adults; they were not independently associated with mortality in this pediatric cohort.¹⁷ Mortality was similar in children with unconfirmed and unlikely tuberculosis. One would expect mortality to be lower in this latter group which includes spontaneous symptom

resolution without anti-tuberculosis treatment as defining criteria. Children who died were much younger and had very advanced HIV disease compared to those alive at M6, showing major heterogeneity in this group. The mortality may be due to other untreated opportunistic or bacterial infections mimicking tuberculosis symptomatology, and mostly to lack of ART initiation.

As expected, anti-tuberculosis treatment was associated with improved survival in both children with confirmed and unconfirmed tuberculosis. However, we did not observe differences between the time to treatment initiation between children who died and those alive at the end of the study. There were very few children with confirmed tuberculosis not receiving anti-tuberculosis treatment as most were confirmed by Xpert. In those classified as unconfirmed tuberculosis, algorithms to improve and accelerate empirical anti-tuberculosis treatment decision are needed, as 60% of those not treated died.

ART was associated with a massive reduction in mortality during the first month of follow-up and not beyond that point. Interpretation of this extreme and immediate effect on survival during the first month must be cautious. Despite adjustment on factors significantly associated to mortality, there may be other confounding factors, either unmeasured or unaccounted for, explaining this higher risk of mortality. This result nonetheless pleads for an early start of ART, consistent with the findings from a study in Malawi which showed a 9-fold increase in mortality in HIV-infected children with tuberculosis not initiated on ART, as compared with those that were started on ART during the first two months of follow-up.²⁰ This seems to contradict a study assessing impact of timing of ART on mortality in South-African children treated for tuberculosis which concluded that ART initiation delayed by 2 to 4 weeks after anti-tuberculosis treatment start did not increase mortality.²¹ However, initial mortality in this study was much lower, possibly masked by a higher rate of loss to follow-up. In our study, the benefit of ART on mortality was not shown in those who survived passed one month, probably because the population on ART included children with the most severe conditions and persistent risk of death under treatment, initiated early during the study, and possibly because of survivor bias.

Anti-tuberculosis treatment led to delayed ART introduction which is a problem in a population with severe clinical status. CD4 below 10%, a threshold associated with high mortality in large pediatric cohorts, was independently associated with mortality in our study,

and sub-group analyses showed higher HIV RNA in those who died. Both plead for an early ART initiation which could be even more challenging in routine program conditions.¹⁹ In children with severity criteria, clear recommendations should therefore be given to shorten time to ART initiation or even initiate it concomitantly with anti-tuberculosis treatment, as it was done for 34% of children from the South-African cohort.²¹ Despite reports of much lower rates of paradoxical IRIS in children than in adults, significant IRIS-related morbidity could be an issue in children.²⁴⁻²⁷

Interestingly, elevated baseline AST was strongly associated with mortality. High AST, present in roughly half of children at inclusion, was not associated with tuberculosis diagnosis but with advanced HIV disease, consistent with our clinical experience. High proportions of elevated transaminases have been reported in ART-naïve HIV-infected Asian children of similar age, CD4 count and nutritional status.²⁸ In severely immunosuppressed HIV-infected Cambodian adults with tuberculosis, elevated AST was associated with a moderate increase in mortality.²⁹ However, to our knowledge, this is the first report showing an association between elevated AST and mortality in HIV-infected children with a suspicion of tuberculosis.

Our study has limitations. First, we used a case definition for tuberculosis which has not been validated in HIV-infected children and which could face the same limitations as the previous NIH consensus definition.³⁰ Classification depends partly on treatment decision and we may have misclassified children between the unconfirmed and unlikely tuberculosis groups. Second, eligibility criteria in our study differ from entry criteria proposed by the updated Intrathoracic Tuberculosis Definitions for Diagnostic Research in Children and WHO's screening criteria to investigate for tuberculosis.^{4,15} Our findings on high mortality and associated factors may not be fully applicable to all children with a suspicion tuberculosis as defined by these approaches. Additionally, the children in this study were fairly old, suggesting longer term survivors and breastfeeding transmission. Tuberculosis diagnostic data and mortality data may not be transferable to the very young children who fail prevention of mother-to-child transmission of HIV or who are diagnosed in the first year of life. At last, this was an observational study, not designed to assess impact of timing of ART and anti-tuberculosis treatment on mortality, and conclusions should be drawn cautiously.

Tuberculosis-associated mortality remains high in HIV-infected children. Improved access to ART with earlier diagnosis and ART initiation before they present with presumptive tuberculosis is needed to prevent many cases of tuberculosis and related mortality. Suspicion of tuberculosis nevertheless remains a frequent and challenging situation in HIV-infected children. Despite the relatively low proportion of bacteriological confirmation, same-day Xpert testing could lead to immediate treatment initiation in children most at risk of dying. Optimized algorithms for empirical anti-tuberculosis treatment decision in those without smear-positive or Xpert-confirmed tuberculosis are needed to reduce mortality and to accelerate initiation of ART. ART initiation remains critical and should not be postponed despite the suspicion of tuberculosis. In children with severity criteria and a high risk of mortality, concomitant initiation of ART and anti-tuberculosis treatment under cautious monitoring should be considered.

RESEARCH IN CONTEXT

Evidence before this study

We searched PubMed for articles published in English before March 31, 2017, with the terms “mortality” and “HIV” and “tuberculosis” and “children” and “associated factors”. Several studies assessed factors associated with mortality, including HIV infection, in pediatric tuberculosis cases. None specifically assessed the question of mortality and associated factors in HIV-infected children with a suspicion of tuberculosis. Two studies specifically assessed the impact of timing of ART initiation on mortality of HIV-infected children treated for tuberculosis. One study from Malawi found that the lack of ART initiation was associated with a 9-fold increase in mortality compared to initiating ART in the first two months of follow-up. The other one, implemented in South Africa, found that ART initiation delayed by 2 to 4 weeks after anti-tuberculosis treatment start did not increase mortality, and that there was a trend toward higher mortality in those with severe immune suppression when ART was postponed by more than 60 days. Other associated factors identified in HIV-infected children on anti-tuberculosis treatment were male sex, severe immune suppression, high HIV RNA, young age, low weight-for-age Z score.

Added value of this study

To our knowledge, this is the first study addressing mortality and associated factors in relation with diagnosis and treatment decision in HIV-infected children with a suspicion of tuberculosis. In this cohort where mortality was high in the first 6 months following suspicion

of tuberculosis, we show that mortality was significantly higher in children with bacteriological confirmation either by culture and Xpert MTB/RIF, as internationally recommended for tuberculosis reference diagnosis, or by Xpert MTB/RIF or smear microscopy, which are immediately available. Introduction of anti-tuberculosis treatment led to decreased mortality in both confirmed and unconfirmed tuberculosis and to delayed ART introduction, which is a problem, as ART was associated with a major reduction in mortality when started during the first month of follow-up. Additional factors associated with mortality identified were young age, CD4<10%, miliary features, and elevated serum transaminases.

Implications of all the available evidence

Our study highlights the need for early ART introduction in HIV-infected children with a suspicion of tuberculosis. ART should not be postponed despite the suspicion of tuberculosis. In this regard optimized algorithms for empirical anti-tuberculosis treatment decision are therefore needed to reduce mortality and accelerate initiation of ART. Whether transaminases which are easily measurable markers should be used to guide quick initiation of ART warrants further research.

CONTRIBUTORS

Olivier Marcy, Mathurin Tejiokem, Philippe Msellati, Boubacar Nacro, Laurence Borand, Christophe Delacourt, and Stéphane Blanche designed and wrote the study protocol. Olivier Marcy and Vibol Ung led the study. Mathurin Tejiokem, Philippe Msellati, and Laurence Borand coordinated study implementation at regional and country level. Huu Khanh Truong, Viet Do Chau, Duong Ngoc Tran, Boubacar Nacro, Francis Ateba-Ndong, Suzie Tetang-Ndiang, Bunnet Dim, Leakhena Neou, and Vibol Ung enrolled patients and implemented the study. Christophe Delacourt and Laureline Berteloot reviewed chest radiographs. Olivier Marcy analyzed the data. Olivier Marcy, Mathurin Tejiokem, Philippe Msellati, Christophe Delacourt, and Stéphane Blanche interpreted the data. Olivier Marcy wrote the first draft of the report and all authors reviewed and approved the final version of the report.

CONFLICTS OF INTEREST

Authors reported no conflict of interest.

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Figure 1. Kaplan–Meier Survival Estimates: A) Overall ART-naïve cohort; B) By tuberculosis reference diagnosis; by anti-tuberculosis treatment intake in C) Children with confirmed tuberculosis,, D) Children with unconfirmed tuberculosis.

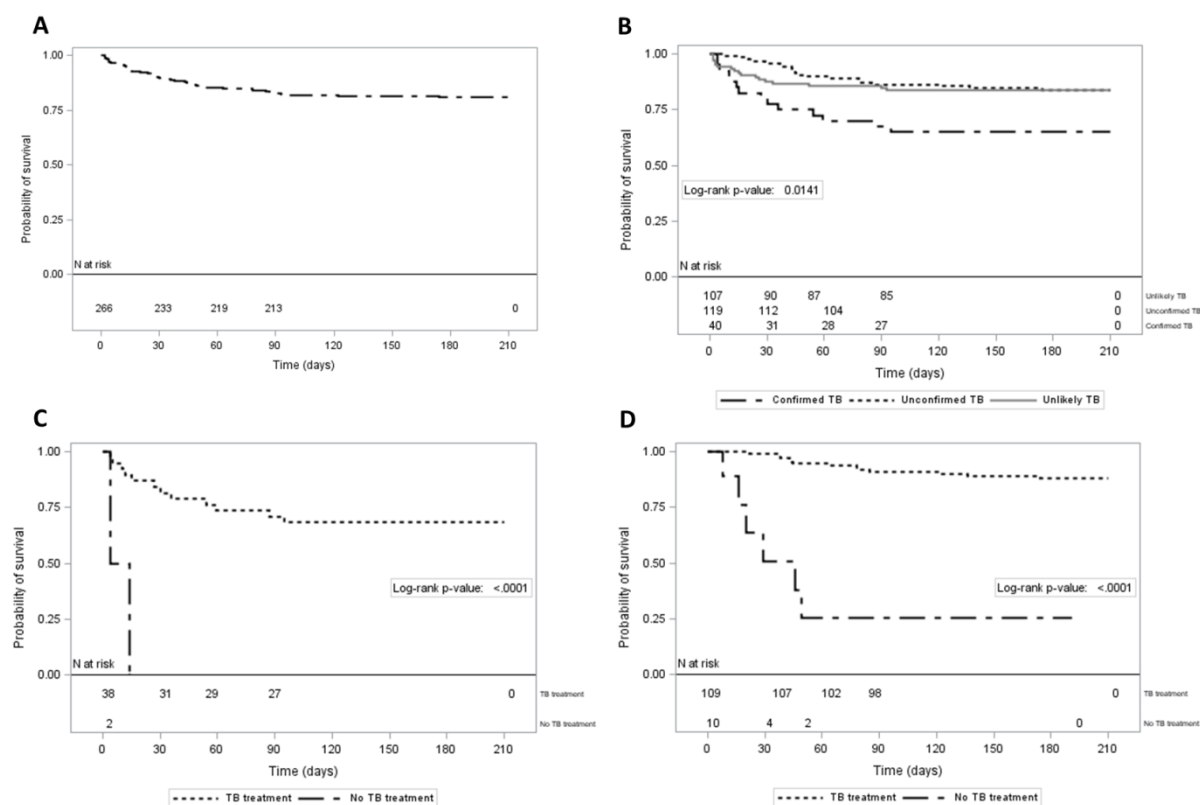


Table 1. Characteristics of children enrolled in the study

	All ART naïve children		Confirmed TB		Unconfirmed TB		Unlikely TB		P-Value
	n*	(N=266)	n*	(N=40)	n*	(N=119)	n*	(N=107)	
Age, years		6.1 (2.3 – 9.1)		7.8 (5.1 – 9.8)		5.9 (2.9 – 9.0)		5.7 (1.7 – 9.3)	0.100
Age categories, years									0.187
<2		61 (22.9)		6 (15.0)		23 (19.3)		32 (29.9)	
[2 – 5[46 (17.3)		4 (10.0)		24 (20.2)		18 (16.8)	
[5 – 10[114 (42.9)		20 (50.0)		53 (44.5)		41 (38.3)	
≥ 10		45 (16.9)		10 (25.0)		19 (16.0)		16 (15.0)	
Sex (male)		137 (51.5)		24 (60.0)		62 (52.1)		51 (47.7)	0.406
Country									<0.0001
Burkina Faso		25 (9.4)		6 (15.0)		8 (6.7)		11 (0.3)	
Cambodia		52 (19.5)		17 (42.5)		36 (30.3)		54 (50.5)	
Cameroon		107 (40.2)		2 (5.0)		40 (33.6)		10 (9.3)	
Vietnam		82 (30.8)		15 (37.5)		35 (29.4)		32 (29.9)	
Underweight (weight for age Z score < – 2)	265	183 (68.8)		28 (70.0)	118	85 (72.0)		70 (65.4)	0.193
Weight for age Z score	265	-2.5 (-3.6 to -1.9)		-2.5 (-3.6 to -1.8)	118	-2.5 (-3.6 to -2.0)		-2.5 (-3.9 to -1.6)	0.605
Weight-for-height Z score (age < 5 y.)	107	-2.0 (-3.6 to -0.8)	10	-3.0 (-3.5 to -2.0)	47	-1.8 (-3.3 to -0.8)	50	-2.1 (-4.0 to -0.7)	0.414
BMI-for-age Z score (age ≥ 5 y.)	157	1.7 (-3.1 to -	29	1.9 (-3.1 to -0.5)	72	-1.7 (-2.9 to -1.0)	56	-1.6 (-3.1 to -0.7)	0.986

				0.8)					
Lansky play performance score	262	80 (70 – 100)		80 (60 - 90)	116	80 (70 - 100)	106	90 (70 - 100)	0.022
Previous TB treatment		13 (4.9)		3 (7.5)		8 (6.7)		2 (1.9)	0.080
Severe [†]		120 (45.3)		28 (70.0)		49 (41.2)		43 (40.2)	0.003
CD4 absolute count (cells per µL)	255	200 (25 – 753)		74 (24 - 379)		176 (26 - 748)	96	333 (24 - 813)	0.276
CD4 percentage	254	9.0 (2.0 – 18.0)		9.0 (2.2 - 16.1)		7.8 (1.5 - 18.0)	95	10.1 (2.0 - 19.7)	0.746
Immune depression [†]	254						95		0.763
Not significant		40 (15.7)		7 (17.5)		20 (16.8)		13 (13.7)	
Mild and advanced		20 (7.9)		2 (5.0)		8 (6.7)		10 (10.5)	
Severe		60 (23.6)		9 (22.5)		25 (21.0)		26 (27.4)	
Very severe (<10%)		134 (52.8)		22 (55.0)		66 (55.5)		46 (48.4)	
Undetectable HIV RNA	260	12 (4.6)		1 (2.5)		7 (5.9)	101	4 (3.7)	0.723
HIV RNA (Log10 copies/mL)	248	5.9 (5.4 – 6.4)	39	6.1 (5.6 - 6.7)	112	5.9 (5.4 - 6.3)	97	5.8 (5.4 - 6.3)	0.176
Hemoglobin (g/dL)	259	9.6 (8.2 – 10.6)		8.2 (6.5 - 9.6)		9.9 (8.2 - 10.8)	100	9.9 (8.7 - 10.7)	<0.0001
ALT (UI/L)	257	34 (20 – 64)		30 (18 - 58)	118	36 (19 - 65)	99	34 (22 - 56)	0.7758
AST (UI/L)	257	48 (36 – 83)		55 (39 - 72)	118	46 (36 - 94)	99	48 (35 - 75)	0.6324
Platelets	259	315 (218 – 426)		293 (240 - 345)		323 (229 - 437)	100	318 (209 - 420)	0.462
Neutrophils	259	3417 (1960 – 5370)		5088 (3052 - 10283)		3450 (2072 - 5775)	100	2768 (1870 - 4075)	<0.0001
Cotrimoxazole prophylaxis at any time in the study		238 (89.5)		38 (95.0)		102 (85.7)		98 (91.6)	0.166
Acid-fast bacilli smear positive	257	24 (9.3)		20 (50.0)		3 (2.5)	98	1 (1.0)	<0.0001
Xpert MTB/RIF positive	257	33 (12.8)		33 (82.5)		0	98	0	

Culture confirmed tuberculosis	257	36 (14.1)		36 (90.0)		0	98	0	
Non-tuberculous mycobacteria positive culture	257	36 (14.1)		4 (10.0)		17 (14.3)	98	15 (15.3)	0.777
Abnormal chest radiograph	251	224 (89.2)	37	35 (94.6)	117	108 (92.3)	97	81 (83.5)	0.062
Chest radiograph consistent with TB	245	180 (73.5)	37	29 (78.4)	115	100 (87.0)	93	51 (54.8)	<0.0001
Miliary feature on CXR	250	17 (6.8)	37	6 (16.2)	116	6 (5.2)	97	5 (5.2)	0.048
Alveolar opacities on CXR	250	143 (57.2)	37	20 (54.1)	117	78 (66.7)	96	45 (46.9)	0.014
Initiated tuberculosis treatment		154 (57.9)		38 (95)		109 (91.6)		7 (6.5)	<0.0001
Time to tuberculosis treatment (days)	154	7 (5 – 11)		6 (3 – 10)		8 (6 – 10)		9 (7 - 46)	0.019
Initiated ART		212 (79.7)		30 (75.0)		104 (87.4)		78 (72.9)	0.019
Time to ART initiation (days)	212	24 (14 – 41)	30	31 (15 – 36)	104	31 (17 – 44)	78	15 (11 – 32)	0.0008

Data are n(%) or median (IQR). ART=antiretroviral treatment. *If n different from N. †Fever >38°C, and/or age-defined tachycardia, and/or age-defined tachypnea.

Table 2. Characteristics of children in the tuberculosis reference diagnosis groups according to their mortality status at 6 months

	Confirmed tuberculosis				Unconfirmed tuberculosis					Unlikely tuberculosis					
	N *	Alive		P- value	N*	Alive		P- value	N*	Alive		P- value			
		(N=26)	N*			(N=14)	(N=97)			N*	(N=19)		(N=83)	N*	(N=17)
Age, years		8.8 (5.9 – 10.8)		5.4 (1.6 – 9.2)	0.106		6.1 (3.3 – 9.0)		3.6 (1.1 – 8.1)	0.116		7.1 (2.2 – 9.4)		1.8 (0.5 – 3.9)	0.005 3
Sex (male)		14 (53.8)		10 (71.4)	0.330		51 (52.6)		8 (42.1)	0.404		38 (45.8)		10 (58.8)	0.327
Weight for age Z score (SD)		-2.5 (-3.3 to -2.0)		-2.2 (-3.7 to -1.4)	0.898		-2.5 (-3.6 to -2.0)	18	-2.6 (-3.8 to -2.3)	0.500		-2.5 (-3.2 to -1.4)		-4.1 (-5.5 to -2.4)	0.008 8
Lansky play performance score		80 (60 – 95)		70 (40 - 90)	0.189	94	80 (80 - 100)		80 (70 - 100)	0.677		90 (70 - 100)		70 (50 - 80)	0.0011
Severe [†]		17 (65.4)		11 (76.6)	0.385		35 (36.1)		12 (63.2)	0.028		36 (43.4)		7 (41.2)	0.868
CD4 percentage		7.9 (2.0 – 17.0)		8.7 (5.3 – 10.8)	0.876		7.0 (1.5 – 18.0)		3.5 (1.0 – 19.0)	0.768	78	10.5 (2.0 – 19.7)	13	6.1 (3.2 – 10.9)	0.373
CD4 <10%		14 (51.9)		10 (66.7)	0.386		54 (55.7)		11 (57.9)	1.000	78	37 (47.4)	13	9 (69.2)	0.146
CD4 absolute count (cells per µL)		74 (27 – 391)		75 (17 – 366)	0.921		227 (28 – 753)		80 (8 – 748)	0.768	78	362 (24 – 858)	13	170 (32 – 324)	0.147
HIV RNA (Log10 copies/mL)	25	5.9 (5.5 – 6.7)		6.4 (6.2 – 6.9)	0.090	91	5.9 (5.3 – 6.3)	18	6.3 (5.8 – 6.5)	0.035	79	5.7 (5.4 – 6.3)	14	6.4 (5.9 – 7.0)	0.002
Hemoglobin (g/dL)		8.2 (6.3 - 9.6)		8.3 (6.8 - 9.5)	0.640		10.1 (8.5 – 10.9)		8.8 (7.5 - 10.3)	0.045	83	9.9 (8.8 - 10.8)	13	9.0 (8.1 - 10.1)	0.157
AST (UI/L)		53 (40 – 62)		66 (35 – 148)	0.0004	96	41.5 (34 – 73)		86 (38 – 187)	0.037	82	44 (35 – 57)	13	90 (78 – 140)	0.0004
Platelets		289 (248 - 352)		306 (232 – 338)	0.955	96	327 (241 - 437)		283 (89 – 397)	0.116	87	326 (220 – 450)	13	260 (140 – 352)	0.138
Cotrimoxazole prophylaxis at inclusion		19 (73.1)		13 (92.9)	0.222		67 (69.1)		16 (84.2)	0.181		65 (78.3)		15 (88.2)	0.512
Culture confirmed tuberculosis		24 (92.3)		12 (85.7)			0		0			0		0	
Smear or Xpert + at baseline		21 (80.8)		12 (85.7)	1.000		2 (2.1)		1 (5.3)	0.418		0		1 (5.9)	0.170

CXR consistent with TB	25	18 (72.0)	12	11 (91.7)	0.306	93	81 (87.1)	16 (84.2)	0.753	80	49 (61.3)	9	1 (11.1)	0.0016
Miliary feature on CXR		1 (3.8)	11	5 (45.5)	0.005	93	5 (5.3)	1 (5.3)	1.000		5 (6.0)	9	0	1.000
Alveolar opacities on CXR		13 (50.0)	11	7 (63.6)	0.495	94	61 (64.2)	14 (73.7)	0.427	82	41 (50.0)	9	2 (22.0)	0.164
Initiated TB treatment		26 (100)		12 (85.7)	0.117		95 (97.9)	13 (68.4)	0.0002		1 (1.2)		5 (29.4)	NA
Time to TB treatment (days)	26	6.5 (3 – 11)	12	5 (2.5 – 8)	0.549	95	8 (6 – 11)	13	7 (5 – 18)	0.913	1	133	5	8 (7 – 9) NA
Initiated ART		24 (92.3)		6 (42.9)	0.0012		91 (93.8)	12 (63.2)	0.0010		70 (84.3)		6 (35.3)	<0.0001
Time to ART initiation (days)	24	32 (15 – 38)	6	18 (14 – 28)	0.154	91	30 (17 – 45)	12	32 (12.5 – 44)	0.696	70	16 (11 – 41)	14	14 (10 – 15) 0.231
Time to end of follow-up (days)		181 (177 - 195)		21 (10 - 54)			182 (175 - 188)		45 (29 -78)		183 (175 - 193)		15 (3 - 39)	

Data are median (IQR) or n (%). BMI=body mass index; TB= tuberculosis; ALT= alanine aminotransferase. AST=aspartate aminotransferase.

CXR= chest radiograph. ART=antiretroviral treatment. *N if different from N reported on top of the column. †Fever >38°C, and/or age-defined tachycardia, and/or age-defined tachypnea.

Table 3. Factors associated with mortality in ART-naïve children (all measured at baseline, except antiretroviral therapy)

	Univariate analysis			Multivariate analysis		
	hazard ratio (95%CI)			hazard ratio (95%CI)		
Tuberculosis reference diagnosis and treatment						
Confirmed tuberculosis	2.448	1.210	4.953	6.331	2.150	18.642
Unconfirmed tuberculosis	0.960	0.494	1.868	1.896	0.789	4.555
Unlikely tuberculosis	1			1		
Antiretroviral Therapy effect during the first month of follow-up*						
No	1			1		
Yes	0.382	0.107	1.360	0.084	0.010	0.670
Antiretroviral Therapy effect after the first month of follow-up*						
No	1			1		
Yes	3.097	0.714	13.425	1.133	0.244	5.271
Age (years)						
<2	1			1		
[2 - 5[0.344	0.148	0.802	0.273	0.095	0.784
[5 - 10[0.231	0.115	0.466	0.201	0.082	0.493
≥10	0.386	0.173	0.864	0.170	0.058	0.495
Country						
BF	1					
CM	3.888	0.928	16.294			
KH	0.959	0.176	5.236			
VN	2.548	0.583	11.144			

Weight for Age Z score (SD)									0.217
≥ -2	1								
[-3 to -2[1.079	0.499	2.333						
[-4 to -3[1.606	0.694	3.717						
< -4	2.070	0.945	4.537						
Severe [†]									0.028
No	1								
Yes	1.888	1.072	3.325						
Lansky score									0.015
100	0.536	0.264	1.090						
]80 – 90]	0.237	0.071	0.783						
]70 – 80]	0.407	0.200	0.827						
≤ 70	1								
Smear or Xpert + at baseline									0.0015
No	1								
Yes	2.717	1.465	5.039						
Non-tuberculous mycobacteria positive culture									0.424
No	1								
Yes	0.686	0.272	1.728						
CD4									0.151
<10%	1.551	0.852	2.823			2.626	1.246	5.534	0.011
$\geq 10\%$	1					1			
HIV RNA (Log10 copies/mL)									0.0008

<5.5	1						
[5.0 – 5.5[1.250	0.403	3.876				
[5.5 – 6.0[4.475	1.776	11.277				
≥ 6.5	4.339	1.697	11.090				
Hemoglobin count (g/dL)				0.084			
<7.0	1						
[7.0 – 9.0[2.026	0.682	6.022				
[9.0 – 11.0[2.898	0.969	8.669				
≥11.0	4.103	1.263	13.327				
AST (ULN)				<0.0001			0.0026
≤ 1.25	1				1		
]1.25 - 2.5]	2.592	1.201	5.593		0.991	0.374	2.629
]2.5 - 5.0]	5.680	2.700	11.948		4.400	1.818	10.654
>5	4.095	1.460	11.486		3.139	0.994	9.915
Platelets				0.0002			
<50 000	5.448	2.119	14.006				
[50 000 - 150 000]	2.759	1.317	5.780				
>150 0000	1						
Miliary features on chest radiograph				0.016			0.0041
No	1				1		
Yes	2.919	1.224	6.957		4.080	1.562	10.658

SD=standard deviation. AST=aspartate amino-transferase. ULN=upper limit of normal. * time dependent variable. †Fever >38°C, and/or age-defined tachycardia, and/or age-defined tachypnea.

4 Performance of Xpert MTB/RIF and alternative specimen collection methods for the diagnosis of tuberculosis in HIV-infected children

4.1 Research question and background

Following endorsement of the Xpert MTB/RIF tests by the WHO in 2011 for the detection of tuberculosis in HIV-infected individuals and detection of multi-drug resistant tuberculosis (61, 241), the GeneXpert platform has been widely distributed in high tuberculosis burden countries, although coverage remains imperfect. The WHO's initial recommendations for the use of Xpert MTB/RIF have been updated and broadened to children in 2013 (242). A meta-analysis on 15 studies in children estimated the pooled sensitivities and specificities of Xpert for culture-confirmed tuberculosis to 62% and 98%, respectively, with use of expectorated or induced sputum samples and 66% and 98%, respectively, with use of samples from gastric aspirates or lavage (64). Expectorated or induced sputum and gastric aspirates/lavage are the samples currently recommended by the WHO (8). Their implementation however remains challenging; expectorated sputum is not feasible in young children, and induced sputum samples as well as gastric aspirates present technical challenges, and are rarely implemented at decentralized level (96). Children therefore do not benefit from the diagnostic revolution of the Xpert MTB/RIF as appropriate pediatric samples are not performed.

Alternative sample collection methods which have shown promising results with other microbiological tests or on preliminary studies with Xpert, including nasopharyngeal aspirates (81, 178, 243), stool samples (88, 93, 244), and the string tests (85, 86), could be of interest to improve access to molecular diagnostic tools such as the Xpert MTB/RIF for HIV-infected children and should be evaluated in this population. Improvement in these methods is crucial, as more progresses are expected soon in molecular diagnosis of MTB, but the chances of a non-sputum based point-of-care test for tuberculosis being available soon are very limited.

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This study has been implemented in the first 281 patients enrolled in the ANRS 12229 PAANTHER 01 study until August 31, 2013. Results have been presented at the Union conference in Paris, France, 2013, at the AFRAVIH conference in Montpellier, France, 2014, and published in *Clinical Infectious Diseases*.

This publication was accompanied by:

1. An editorial commentary by Anne Detjen and Elisabetta Walters (245). It is presented in Appendix 2 of this thesis.
2. A supplementary appendix that has been made available for readers. It is presented in Appendix 3.

Performance of Xpert MTB/RIF and Alternative Specimen Collection Methods for the Diagnosis of Tuberculosis in HIV-Infected Children

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(See the Editorial Commentary by Detjen and Walters on pages 1169–71.)

Background. The diagnosis of tuberculosis in human immunodeficiency virus (HIV)-infected children is challenging. We assessed the performance of alternative specimen collection methods for tuberculosis diagnosis in HIV-infected children using Xpert MTB/RIF (Xpert).

Methods. HIV-infected children aged ≤ 13 years with suspected intrathoracic tuberculosis were enrolled in 8 hospitals in Burkina Faso, Cambodia, Cameroon, and Vietnam. Gastric aspirates were taken for children aged < 10 years and expectorated sputum samples were taken for children aged ≥ 10 years (standard samples); nasopharyngeal aspirate and stool were taken for all children, and a string test was performed if the child was aged ≥ 4 years (alternative samples). All samples were tested with Xpert. The diagnostic accuracy of Xpert for culture-confirmed tuberculosis was analyzed in intention-to-diagnose and per-protocol approaches.

Results. Of 281 children enrolled, 272 (96.8%) had ≥ 1 specimen tested with Xpert (intention-to-diagnose population), and 179 (63.5%) had all samples tested with Xpert (per-protocol population). Tuberculosis was culture-confirmed in 29/272 (10.7%) children. Intention-to-diagnose sensitivities of Xpert performed on all, standard, and alternative samples were 79.3% (95% confidence interval [CI], 60.3–92.0), 72.4% (95% CI, 52.8–87.3), and 75.9% (95% CI, 56.5–89.7), respectively. Specificities were $\geq 97.5\%$. Xpert combined on nasopharyngeal aspirate and stool had intention-to-diagnose and per-protocol sensitivities of 75.9% (95% CI, 56.5–89.7) and 75.0% (95% CI, 47.6–92.7), respectively.

Conclusions. The combination of nasopharyngeal aspirate and stool sample is a promising alternative to methods usually recommended by national programs. Xpert performed on respiratory and stools samples enables rapid confirmation of tuberculosis diagnosis in HIV-infected children.

Clinical Trials Registration. The ANRS (Agence Nationale de Recherche sur le Sida) 12229 PAANTHER (Pediatric Asian African Network for Tuberculosis and HIV Research) 01 study is registered at ClinicalTrials.gov (NCT01331811).

Keywords. tuberculosis; children; HIV infection; diagnosis; Xpert MTB/RIF.

In 2014, the World Health Organization (WHO) estimated the annual burden of childhood tuberculosis to be 1 million cases and 140 000 deaths, representing 10% and 9% of global caseload and mortality, respectively [1]. These updated figures, which are based on recent modeling and statistical estimates, highlight the gap between the global caseload and cases reported to WHO

[2, 3]. Childhood tuberculosis is indeed underdiagnosed and underreported due to the challenging diagnostic confirmation that is inherent in the paucibacillary nature of the disease and the difficulty in obtaining expectorated sputum from children [4].

Diagnosis is even more challenging in human immunodeficiency virus (HIV)-infected children due to the low specificity of clinical and radiological features and the low sensitivity of tuberculin skin tests that are used in various scores and diagnostic approaches [5]. These difficulties occur in a context of more severe clinical presentations, co-treatment issues and challenges, and higher mortality, which further justify rapid diagnosis and treatment [6, 7].

WHO endorsed the automated nucleic acid amplification test (NAAT) Xpert MTB/RIF (Xpert; Cepheid, Sunnyvale, California)

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in 2010 for the diagnosis of HIV-associated and drug-resistant tuberculosis in adults [8, 9]. A recent metaanalysis showed its performance for rapid diagnosis of culture-confirmed pulmonary tuberculosis in children [10]. These findings support the revised WHO recommendations on scaling-up the use of Xpert in children [11]. However, in children, not only the assay but also the specimen collection methods, their feasibility, and their ability to retrieve bacilli are key factors that contribute to the microbiological diagnosis of tuberculosis. Standard samples recommended by WHO include expectorated sputum, which is not feasible in young children; gastric aspirate, which requires fasting and, most often, hospitalization; and induced sputum [11–13]. To date, the implementation of methods to collect samples in children unable to expectorate remains very limited. Of 651 pediatric HIV sites surveyed in sub-Saharan Africa in 2012, only 6% had the capacity to sample induced sputum and only 5% had the capacity to sample gastric aspirates, while 87% had smear microscopy and 54% had mycobacterial culture capacities [14]. As Xpert is rolled out in national programs, the limited capacity to implement specimen collection in children restricts the impact of the near point-of-care introduction of the Xpert assay [9, 15, 16].

Here, we determined whether the use of Xpert with a combination of alternative specimen collection methods not yet recommended by WHO or national programs, including nasopharyngeal aspirates, a string test, and stool samples, would be effective to diagnose tuberculosis in HIV-infected children.

METHODS

Study Design and Participants

We conducted a prospective cohort study in 8 hospitals in Burkina Faso, Cambodia, Cameroon, and Vietnam (see [Supplementary Appendix](#)). From April 2011 to August 2013, HIV-infected children aged ≤ 13 years with a suspicion of intrathoracic tuberculosis based on at least 1 of the following: persistent cough; persistent fever; failure to thrive, defined as recent deviation in the growth curve or a weight-for-age Z score < -2 standard deviation; failure of broad spectrum antibiotics for a pulmonary infection; or suggestive chest radiograph anomaly were enrolled in the study after receipt of parent or guardian informed consent. Children with a history of tuberculosis treatment started within 2 years prior to the study were not eligible.

Each country's national ethics committee or relevant institutional review board approved the study.

Procedures

All children underwent a complete physical exam, a chest radiograph with standard anteroposterior view for all ages and an additional lateral view for children aged < 5 years, a baseline blood sample including HIV viral load and CD4 count, and a tuberculin skin test.

Bacteriological samples were taken using standard collection methods that consisted of 2 gastric aspirates in children aged < 10 years and 3 expectorated sputum samples in those aged ≥ 10 years, as well as alternative methods that involved the collection of 1 nasopharyngeal aspirate and 1 stool sample for all ages and 1 string test for those aged ≥ 4 years. For children aged < 4 years, the string test was replaced by collection of a third gastric aspirate. Trained nurses collected specimens in the early morning after overnight fasting, using standardized procedures (see [Supplementary Appendix 3](#)). Gastric aspirates were neutralized with 8% sodium bicarbonate. A gastric lavage with 10 mL sterile water was performed if spontaneous aspirate could not be obtained. Nasopharyngeal aspiration was performed without prior nasal instillation using a mucus aspirator connected to a suction device in order to stimulate the cough reflex and obtain 2–5 mL of mucus [16]. The string test was performed using the pediatric Entero-Test (HDC Corporation, Milpitas, California) [17, 18]. The gelatin capsule was swallowed, unraveling a 90-cm absorbent nylon string, and the non-absorbent part of the string was taped to the cheek with plaster. After an intragastric downtime of 2 hours, the string was retrieved and the absorbent part was deposited in a Falcon tube containing 3 mL 0.9% saline.

Samples were transported in ice chests to laboratories within 4 hours of collection. All samples were decontaminated with N-acetyl-L-cysteine and sodium hydroxide and centrifuged; stool samples were previously prepared by dilution in sterile water. Centrifuged pellets were resuspended in phosphate buffer. Direct exam was performed using the Ziehl–Neelsen method for stool samples and fluorescent (auramine) microscopy from a drop of pellet suspension was performed for all other samples. Sample pellet suspensions were inoculated in Lowenstein–Jensen slants and/or cultured using automated liquid culture (Bactec MGIT; Becton Dickinson, Maryland). Isolates were identified as *Mycobacterium tuberculosis* complex by Ziehl–Neelsen staining and biochemical method, immunochromatographic tests, or commercial multiplex line-probe assays (see [Supplementary Appendix](#)).

The Xpert assay was performed on 1.0 mL of fresh sample or decontaminated pellet if the sample volume was not sufficient, following the manufacturer's recommendations. Stool samples were previously processed by emulsification of 0.5 g of material in Sheather's solution (28% sucrose in distilled water), filtered through funnel gauze, and centrifuged (see [Supplementary Appendix](#)). Because Xpert was not available at study onset at 2 sites, the assay was retrospectively performed on frozen samples for some of the children.

Xpert results for all samples, if performed prospectively, were available to the treating physician for diagnosis. All children were followed for 6 months during the study, with visits at 1, 2, 3, and 6 months following inclusion. Antiretrovirals and antituberculosis treatment were initiated per national guidelines and provided by national programs.

Statistical Analysis

We used culture on any of the samples as the reference standard for calculation of sensitivity and specificity. In addition, we classified tuberculosis using the Intrathoracic Tuberculosis Definitions for Diagnostic Research in Children as confirmed, probable, or possible tuberculosis or tuberculosis unlikely and not tuberculosis [19]. Chest radiographs were reviewed independently by 2 readers blinded to clinical and biological data; discordant opinions were resolved by a third reader.

We analyzed the data using both the intention-to-diagnose approach, considering any child with at least 1 Xpert performed on any specimen, and the per-protocol approach, restricting analyses to children with Xpert performed per protocol on all specimens [20]. We assessed the diagnostic accuracy of Xpert for confirmed tuberculosis by measuring sensitivity and specificity and their exact binomial 95% confidence intervals (CIs) for different sample combinations. Nonevaluable results included missing tests, either because the specimen was not collected or the test itself was not performed, and indeterminate tests results. In the intention-to-diagnose analysis, we considered

nonevaluable results as negative. We compared performance of Xpert on alternative specimens and the standard specimen using McNemar's χ^2 test. All analyses were performed using Stata 13 software (StataCorp, College Station, Texas).

RESULTS

Study Population

We enrolled 281 children, 272 (96.8%) of whom had at least 1 sample collected and tested with Xpert (intention-to-diagnose population; Figure 1). The median age was 7.2 years (interquartile range, 4.1–7.2), and 186 (68.4%) were underweight (Table 1).

Nurses were able to collect 163/192 (84.9%) planned sputum samples, 469/483 (97.1%) gastric aspirates, 268/272 (98.5%) nasopharyngeal aspirates, and 267/272 (98.2%) stool samples; 162/205 (79.0%) samples were collected with the string test; and 55 additional gastric aspirates were collected in children unable to expectorate sputum or perform the string test, for a total of 1384 samples (see [Supplementary Appendix 5](#)). Of 272 children, 179 (65.8%) had all samples evaluated with Xpert per protocol (Figure 1). Of the 93 children without

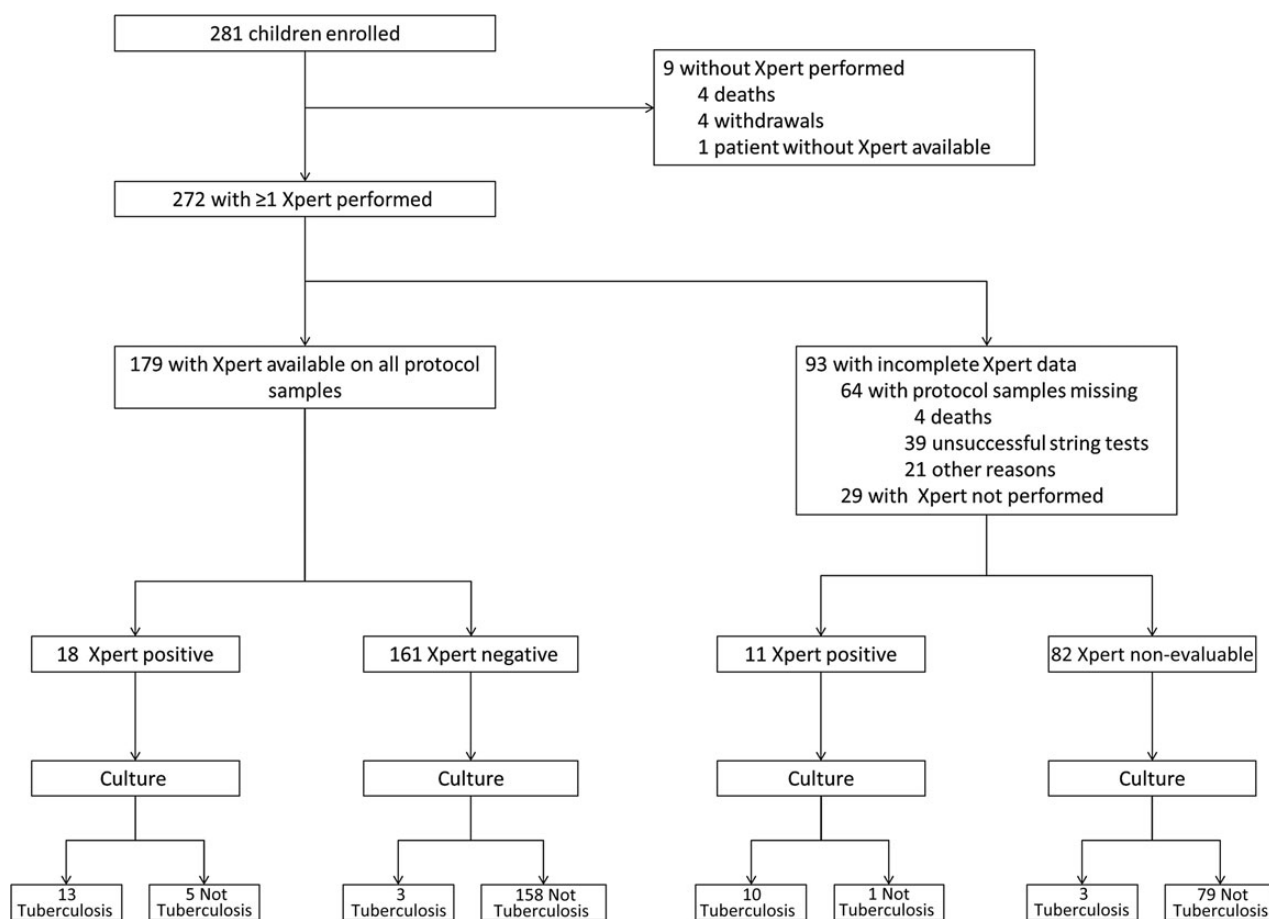


Figure 1. Study profile. Nonevaluable Xpert results are defined as Xpert results missing either because the specimen was not collected or the test itself was not performed, resulting in indeterminate tests results. Tuberculosis refers to culture-confirmed tuberculosis. Not tuberculosis refers to culture negative for tuberculosis, including probable and possible tuberculosis.

Table 1. Baseline Characteristics

Characteristic	All Patients (N = 272)	Tuberculosis Confirmed (N = 29)	Tuberculosis Probable (N = 116)	Tuberculosis Possible (N = 100)	Tuberculosis Unlikely and Not Tuberculosis (N = 27)
Age	7.2 (4.1–7.2)	7.5 (5.6–10.0)	7.2 (3.7–9.5)	7.3 (4.1–9.6)	6.7 (3.4–10.4)
0 to <4 y	67 (24.6)	4 (13.8)	31 (26.7)	24 (24.0)	8 (29.6)
4 to <10 y	141 (51.8)	17 (58.6)	61 (52.6)	53 (53.0)	10 (37.0)
10 y +	64 (23.5)	8 (27.6)	24 (20.7)	23 (23.0)	9 (33.3)
Sex (female)	132 (48.5)	14 (48.3)	57 (49.1)	50 (50.0)	11 (40.7)
Underweight (weight-for-age Z score <−2) ^a	186 (68.4)	19 (65.5)	87 (75.0)	62 (62.0)	18 (66.7)
Weight-for-age Z score ^a	−2.5 (−3.3 to −1.6)	−2.4 (−3.1 to −1.4)	−2.5 (−3.2 to −1.9)	−2.5 (−3.2 to −1.5)	−2.5 (−4.0 to −1.5)
Height-for-age Z score ^b	−2.3 (−3.3 to −1.5)	−1.9 (−3.1 to −0.9)	−2.5 (−3.4 to −1.7)	−2.1 (−3.3 to −1.4)	−2.2 (−3.5 to −1.6)
Weight-for-height Z score (age <5 y) ^c	−1.8 (−3.3 to −0.4)	−3.2 (−3.4 to −2.0)	−1.2 (−3.2 to −0.3)	−1.7 (−3.0 to −0.5)	−2.6 (−4.3 to −1.2)
Body mass index-for-age Z score (age ≥5 y) ^d	−1.6 (−2.7 to −0.7)	−1.7 (−3.0 to −0.6)	−1.6 (−2.8 to −0.8)	−1.5 (−2.4 to −0.7)	−2.1 (−3.9 to −1.2)
BCG vaccine done	200 (73.5)	23 (79.1)	86 (74.1)	72 (72.0)	19 (70.4)
Previous tuberculosis disease	49 (18.0)	3 (10.3)	31 (26.7)	14 (14.0)	1 (3.7)
On ART at inclusion ^a	121 (44.5)	6 (20.7)	58 (50.0)	49 (49.0)	8 (29.6)
Time on ART (y) ^e	2.4 (0.9–5.1)	1.8 (0.9–4.8)	2.9 (0.2–4.3)	2.9 (1.8–5.7)	1.9 (0.4–6.4)
CD4 absolute count ^f	498 (58–1068)	155 (28–836)	607 (100–1105)	569.5 (60.5–1129.5)	146.5 (16–757)
CD4 percentage ^b	14.5 (4.0–24.0)	11.7 (3.3–24.9)	17.0 (5.3–25.0)	15.5 (3.2–24.5)	5 (2.0–17.1)
Undetectable human immunodeficiency virus RNA	79 (29.0)	3 (10.3)	33 (28.4)	40 (40.0)	3 (11.1)
Hemoglobin (g/L) ^a	10.5 (8.9–11.8)	8.4 (7.7–9.9)	10.6 (9.1–12.0)	10.6 (8.7–11.9)	10.6 (9.6–11.7)
Started on tuberculosis treatment (at M0) ^g	155 (57.01)	24 (82.8)	83 (71.6)	41 (41.0)	7 (25.9)

Data are median (interquartile range) or n (%).

Abbreviation: ART, antiretroviral treatment.

^a Available for 271 children.

^b Available for 265 children.

^c Available for 78 children.

^d Available on 192 children.

^e In 121 children on ART.

^f Available for 270 children.

^g Available for 268 children.

complete Xpert results, 4 died before completion of specimen collection, 29 had all samples collected but Xpert not performed, and 60 had at least 1 sample not collected, including 30 who were unable to swallow the string for the string test

and 9 for whom this procedure was unsuccessful due to vomiting.

Smear microscopy was positive on at least 1 sample in 18 (6.6%) children, of whom 14 of smear positive had culture-confirmed

Table 2. Detailed Bacteriological Results

Samples or Children	N	Culture Tuberculosis Positive			Culture Tuberculosis Negative		
		Smear Positive n (%)	Smear Negative n (%)	Total n (%)	Smear Positive n (%)	Smear Negative n (%)	Total n (%)
Samples							
All	1384	41 (3.0)	55 (4.0)	96 (6.9)	10 (0.7)	1278 (92.3)	1288 (93.1)
Gastric aspirate	524	18 (3.4)	19 (3.6)	37 (7.1)	1 (0.2)	486 (92.7)	487 (92.9)
Sputum sample	163	5 (3.1)	9 (5.5)	14 (8.6)	3 (1.8)	146 (89.6)	149 (91.4)
Nasopharyngeal aspirate	268	7 (2.6)	15 (5.6)	22 (8.2)	1 (0.4)	245 (91.4)	246 (91.8)
String test	162	4 (2.5)	6 (3.7)	10 (6.2)	0 (0.0)	152 (93.8)	152 (93.8)
Stool sample	267	7 (2.6)	6 (2.2)	13 (4.9)	5 (1.9)	249 (93.3)	254 (95.1)
Children							
All	272	14 (5.1)	15 (5.5)	29 (10.7)	4 (1.5)	239 (87.9)	243 (89.3)
Aged <4 y	67	1 (1.5)	3 (4.5)	4 (6.0)	0 (0.0)	63 (94.0)	63 (94.0)
Aged 4–9 y	141	8 (5.7)	9 (6.4)	17 (12.1)	2 (1.4)	122 (86.5)	124 (87.9)
Aged ≥10 y	64	5 (7.8)	3 (4.7)	8 (12.5)	2 (3.1)	54 (84.4)	56 (87.5)

Table 3. Diagnostic Accuracy of Xpert MTB/RIF for Culture-Confirmed Tuberculosis: Intention-to-Diagnose Population (n = 272)

Samples	Xpert Positive		Xpert Non Evaluable ^a		Xpert Negative		Sensitivity % (95% CI)	Specificity % (95% CI)	Sensitivity in Smear- Positive Culture- Confirmed Tuberculosis n/N, % (95% CI)	Sensitivity in Smear- Negative Culture- Confirmed Tuberculosis n/N, % (95% CI)
	Tuberculosis ^b n	Not Tuberculosis ^c n	Tuberculosis ^b n	Not Tuberculosis ^c n	Tuberculosis ^b n	Not Tuberculosis ^c n				
All	23	6	3	79	3	158	79.3 (60.3–92.0)	97.5 (94.7–99.1)	14/14, 100 (76.8–100)	9/15, 60.0 (32.3–83.7)
Standard samples	21	4	5	32	3	207	72.4 (52.8–87.3)	98.4 (95.8–99.5)	14/14, 100 (76.8–100)	7/15, 46.7 (21.3–73.4)
Alternative samples	22	3	1	62	6	178	75.9 (56.5–89.7)	98.8 (96.4–99.7)	14/14, 100 (76.8–100)	8/15, 53.3 (26.6–78.7)
NPA only	20	1	1	12	8	230	69.0 (49.2–84.7)	99.6 (97.7–100)	12/14, 85.7 (57.2–98.2)	8/15, 53.3 (26.6–78.7)
String test only ^d	9	2	9	46	7	132	36.0 (18.0–57.5)	98.9 (96.0–99.9)	9/13, 69.2 (38.6–90.9)	0/12, 0 (0–26.5)
Stool only	18	1	2	19	9	223	62.1 (42.3–79.3)	99.6 (97.7–100)	12/14, 85.7 (57.2–98.2)	6/15, 40.0 (16.3–67.7)
NPA and stool	22	2	1	23	6	218	75.9 (56.5–89.7)	99.2 (91.7–99.9)	14/14, 100 (76.8–100)	8/15, 53.3 (26.6–78.7)
NPA and string test ^d	18	2	1	54	6	124	72.0 (50.6–87.9)	98.9 (96.0–99.9)	13/13, 100 (75.3–100)	5/12, 41.7 (15.2–72.3)
String tests and stool ^d	16	2	2	53	7	125	64.0 (42.5–82.0)	98.9 (96.0–99.9)	12/13, 92.3 (64.0–99.8)	4/12, 33.3 (9.9–65.1)

Abbreviations: CI, confidence interval; NPA, nasopharyngeal aspirate.

^a Nonevaluable Xpert results are defined as Xpert results missing either because the specimen was not collected or the test was not performed, resulting in indeterminate tests results.

^b Culture-confirmed tuberculosis.

^c Culture-negative for tuberculosis.

^d Age ≥4 years only.

Table 4. Diagnostic Accuracy of Xpert MTB/RIF for Culture-Confirmed Tuberculosis: Per-Protocol Population (n = 179)

Samples	Xpert Positive		Xpert Negative		Sensitivity % (95% CI)	Specificity % (95% CI)	Sensitivity in Smear-Positive Culture-Confirmed Tuberculosis n/N, % (95% CI)	Sensitivity in Smear-Negative Culture-Confirmed Tuberculosis n/N, % (95% CI)
	Tuberculosis ^a n	Not Tuberculosis ^b n	Tuberculosis ^a n	Not Tuberculosis ^b n				
All	13	5	3	158	81.3 (54.4–96.0)	96.9 (93.0–99.0)	10/10, 100 (69.2–100)	3/6, 50.0 (11.8–88.2)
Standard samples	13	3	3	160	81.3 (54.4–96.0)	98.2 (94.7–99.6)	10/10, 100 (69.2–100)	3/6, 50.0 (11.8–88.2)
Alternative samples	12	2	4	161	75.0 (47.6–92.7)	98.8 (95.6–99.9)	10/10, 100 (69.2–100)	2/6, 33.3 (4.3–77.7)
NPA only	10	0	6	163	62.5 (35.4–84.8)	100 (97.8–100)	8/10, 80.0 (44.4–97.5)	2/6, 33.3 (4.3–77.7)
String test only ^c	9	1	4	107	69.2 (38.6–90.9)	99.1 (94.9–100)	9/9, 100 (66.4–100)	0/4, 0 (0–60.2)
Stool only	11	1	5	162	68.8 (41.3–89.0)	99.4 (96.6–100)	9/10, 90.0 (55.5–99.7)	2/6, 33.3 (4.3–77.7)
NPA and/or stool	12	1	4	162	75.0 (47.6–92.7)	99.4 (96.6–100)	10/10, 100 (69.2–100)	2/6, 33.3 (4.3–77.7)
NPA and/or string test ^c	9	1	4	107	69.2 (38.6–90.9)	99.1 (94.9–100)	9/9, 100 (66.4–100)	0/4, 0 (0–60.2)
String tests and/or stool ^c	9	1	4	107	69.2 (38.6–90.9)	99.1 (94.9–100)	9/9, 100 (66.4–100)	0/4, 0 (0–60.2)

Abbreviations: CI, confidence interval; NPA, nasopharyngeal aspirate.

^a Culture-confirmed tuberculosis.

^b Culture-negative for tuberculosis.

^c Age ≥4 years only.

tuberculosis. Overall, 29 (10.7%) children had culture-confirmed tuberculosis (Table 2), independently from smear status. In addition, 116 (42.6%) children were classified as having probable tuberculosis, 100 (36.8%) as having possible tuberculosis, and 27 (9.9%) as having tuberculosis unlikely or not tuberculosis (Table 1; see [Supplementary Appendix, Table 3](#)). Of 268 (98.5%) children in follow-up after the diagnosis process, 155 (57.8%) initiated tuberculosis treatment.

Xpert was performed on 1334 (96.4%) samples in 272 children. It was indeterminate in 17 (1.3%) samples and positive in 106 (7.9%) samples from 29 (10.7%) children.

Xpert performed on all samples detected 23 (79.3%) of 29 confirmed tuberculosis cases (Table 3), 3 (2.6%) of 116 probable tuberculosis cases, 2 (2.0%) of 100 possible tuberculosis cases, and 1 (3.7%) of 27 tuberculosis unlikely or not tuberculosis cases (Table 5). The intention-to-diagnose sensitivity of Xpert was 23/29 (79.3%; 95% CI, 60.3–92.0) overall, 14/14 (100%; 95% CI, 76.8–100) in smear-positive and 9/15 (60.0%; 95% CI, 32.3–83.7) in smear-negative culture-confirmed tuberculosis (Table 3). The characteristics of the 6 Xpert-positive culture-negative children are shown in the [Supplementary Appendix, Table 5](#).

Performed on alternative or standard samples, Xpert yielded an identical number of 25 cases. The sensitivity of Xpert on alternative samples (22/29; 75.9%; 95% CI, 56.5–89.7) did not differ significantly from that obtained on standard samples (21/29; 72.4%; 95% CI, 52.8–87.3, $P = .08$). Paired nasopharyngeal aspirate and stool samples were collected in 248 (91.2%) children, and at least 1 of the 2 samples was collected in 272 (100%) children; with this combination, 22 culture-confirmed cases were detected. Compared with nasopharyngeal aspirates, Xpert on stools had an incremental sensitivity of 10% (20/29 to 22/29) and an incremental yield of 14% (21 to 24). Xpert on the string test had no incremental sensitivity but an incremental yield of 4.5% (24 to 25 cases detected).

In the per-protocol population, the sensitivity of Xpert was 81.3% (95% CI, 54.4–96.0) (Table 4). Xpert performed similarly on alternative and standard samples (sensitivity: 12/16; 75.0%; 95% CI, 47.6–92.7 vs 13/16; 81.3%; 95% CI, 54.4–96.0; $P = .31$), with a specificity above 98% overall.

Of 29 children with confirmed tuberculosis, 1 had rifampin resistance that was not detected by Xpert. Conversely, Xpert

detected rifampin resistance in 1 child who remained culture negative and was considered cured on first-line tuberculosis treatment.

Xpert results were available at the time of diagnosis in 130 (48.5%) of 268 children. Xpert was positive in 17 patients, all diagnosed as having tuberculosis by a clinician prior to the availability of results. Among children with Xpert performed secondarily, 4 were diagnosed and initiated tuberculosis treatment after receipt of positive Xpert results. Two children had positive Xpert results but died before treatment initiation.

DISCUSSION

In the PAANTHER study, Xpert used on a combination of nasopharyngeal aspirate, stool sample, and string test had diagnostic performance that was similar to that for Xpert used on either gastric aspirate or expectorated sputum sample, which is usually recommended by national tuberculosis programs. This is the first multicenter study to assess Xpert on a large number of respiratory specimens from different sites and stool samples exclusively in HIV-infected children who present specific diagnostic and therapeutic challenges. We analyzed our results in an intention-to-diagnose approach, which provided better estimates of the operational sensitivity of the proposed strategies compared with a per-protocol approach; this is a key issue in this challenging population [21].

With an overall sensitivity of 79% for Xpert used on all samples and similar sensitivities on alternative and standard sample combinations, we show that Xpert performs equally well in HIV-infected children compared with HIV-uninfected children [10]. Our results are consistent with the 70% pooled sensitivity in HIV-infected children found by the metaanalysis of 15 studies on respiratory samples [10]. Xpert detected all smear-positive culture-confirmed tuberculosis cases and 60% of smear-negative culture-confirmed cases, which is in line with the pooled sensitivity results from the metaanalysis. There was a high proportion (14/29, 48%) of smear-positive culture-confirmed tuberculosis in these HIV-infected children, which may reflect more severe forms of tuberculosis and higher bacterial loads. Children enrolled in the study had severe clinical presentations, with more than two-thirds of children underweight, and there were early deaths. In the group with samples missing, some due to early death, the culture yield was higher than in the

Table 5. Results of Xpert MTB/RIF in the Different Diagnostic Categories

Xpert Results	All Patients (n = 272) n (%)	Tuberculosis Confirmed (n = 29) n (%)	Tuberculosis Probable (n = 116) n (%)	Tuberculosis Possible (n = 100) n (%)	Tuberculosis Unlikely and Not Tuberculosis (n = 27) n (%)
Xpert positive	29 (10.7)	23 (79.3)	3 (2.6)	2 (2.0)	1 (3.7)
Xpert nonevaluable ^a	82 (30.1)	3 (10.3)	30 (25.9)	34 (34.0)	15 (55.6)
Xpert negative	161 (59.2)	3 (10.3)	83 (71.6)	64 (64.0)	11 (40.7)

^a Nonevaluable Xpert results are defined as Xpert results missing either because the specimen was not collected or the test itself was not performed, resulting in indeterminate tests results. All children with nonevaluable Xpert results had at least 1 Xpert-negative result.

per-protocol group, highlighting the importance of rapid and adapted testing strategies in this population with severe clinical features. The specificity of Xpert was very high overall, which reflects its inability to detect *Mycobacterium tuberculosis* in culture-negative children and is a current limitation of the assay [10]. Xpert nevertheless detected tuberculosis in 3 culture-negative symptomatic probable and possible tuberculosis cases.

The performance of Xpert with alternative samples was identical to that with standard samples, with similar overall yield (25 cases) and sensitivity for culture-confirmed tuberculosis. This suggests that these samples have a potential to replace or complement standard samples if standard samples are challenging to implement, for example, collection of sputum samples in young children or collection of gastric aspirates. For both types of specimen, overall performance was based on incremental yield and sensitivity of multiple samples.

To our knowledge, this is the first study to show large-scale results of stool testing with Xpert for the diagnosis of intrathoracic tuberculosis after 2 pilot studies from South Africa [22,23]. Stool samples make it possible to retrieve bacilli from respiratory origin; their interest in tuberculosis diagnosis has been shown both with culture and other NAATs [24–26]. Xpert performed on 1 stool sample had intention-to-diagnose and per-protocol sensitivities of 62.1% and 68.8%, respectively, which confirmed promising results from the 2 South African studies. As shown previously, culture yield in stool was low, which may be due to decontamination methods and rates of contaminated cultures [25,27]. Use of a flotation method based on sucrose, an inexpensive and easily accessible consumable, may have contributed to the performance of Xpert on stool samples.

Despite previous findings that showed that the string test performed better than induced sputum for smear-negative HIV-infected adults [18] and had a very good acceptability in children aged ≥ 4 years [17], its performance in our study was disappointing. Its per-protocol sensitivity was 69%; however, a high failure rate hampered its overall performance. It detected none of the smear-negative culture-confirmed cases but had a 4.5% incremental yield by detecting 1 additional culture-negative child.

Conversely, nasopharyngeal aspirate had excellent feasibility and good diagnosis accuracy when tested with Xpert. Previous studies showed that it had similar sensitivity to induced sputum despite lower culture yield [28,29]. In our study, its sensitivity as a single test was similar to the 70% pooled sensitivity found by the metaanalysis in HIV-positive children and slightly better than previous studies, probably due to the high smear-positivity rate. This well-tolerated method is of particular interest as it does not require a child to fast, unlike gastric aspirates, and is better tolerated than induced sputum in children with severe respiratory deficit [16]. WHO recommended Xpert as a replacement test for tuberculosis diagnosis in children in 2013 but gave no clear recommendations on the optimal specimen collection strategy [30]. In our study, 100% of children had at least 1

nasopharyngeal aspirate or 1 stool sample performed, and this combination had sensitivity (75.9%) and yield (24 cases) that were similar to those of standard specimens. Both methods could be recommended for large-scale implementation of child-friendly methods for performance of NAATs in children unable to expectorate sputum, as routine implementation of gastric aspirates remains poorly accepted and induced sputum is challenging [14]. Furthermore, collection of nasopharyngeal aspirate and stool sample is suitable for younger children [23,28]. However, at this stage, stool processing for Xpert is complex and time consuming; an optimized centrifugation-free process for stool preparation would facilitate its implementation at the near point-of-care level. Whether our results apply to a predominantly HIV-uninfected population warrants further studies as they may present with lower proportions of smear-positive tuberculosis [10].

There are limitations to our study. First, in terms of a reference standard, heterogeneity in culture methods led to differential verification; availability of solid culture only in Burkina Faso may have contributed to a higher rate of culture-negative Xpert-positive cases. Use of case definitions not specifically developed for HIV-infected children may also have led to the high rate of probable and possible tuberculosis in our study population [19]. Second, Xpert was performed retrospectively in some of the children and was therefore not always available to clinicians, which may explain why children with positive Xpert results were classified as possible tuberculosis if they were not initially started on treatment. Finally, we chose not to test induced sputum due to practical challenges in the nebulization process and concerns about nosocomial transmission and therefore were unable to compare results from alternative samples with those of induced sputum, which showed consistently high yields both with culture and NAAT [29,31]. Xpert was, however, equally effective with induced sputum and with expectorated sputum and gastric aspirates, which were used as standard samples in our study [10].

Due to the nature of childhood tuberculosis, microbiological confirmation has its limitation. The accuracy of Xpert in children is still suboptimal compared with its performance in adults [10,32]. In our study, Xpert detected only 26 of 145 confirmed and probable tuberculosis cases. It primarily confirmed clinical diagnosis but also made it possible to diagnose tuberculosis that had been undetected on routine exams in 4 children. The ongoing development of new NAATs such as Xpert Ultra with an expected lower limit of detection could impact significantly the capacity to reach diagnosis confirmation. An empirical treatment decision based on clinical and radiological features remains frequent in children but challenging in the context of HIV infection due to other opportunistic infections or diseases. Other point-of-care tests and algorithmic approaches are therefore needed in all children, especially in those infected with HIV, in order to help clinicians.

Supplementary Data

Supplementary materials are available at <http://cid.oxfordjournals.org>. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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5 Value of Chest X Ray in the diagnosis of tuberculosis in HIV-infected children living in resource limited countries. Results from the ANRS 12229 - PAANTHER 01 study

5.1 Research question and background

CXR plays a major role in diagnosis of tuberculosis in children (246). Its findings are also used widely to define cases in reference standards for diagnostic studies (28, 100, 179). Despite known challenges in interpretation (246), including detecting perihilar lymphadenopathies in young children (247), CXR is part of routine clinical practice as a screening or a diagnosis. In HIV-infected children, specificity of CXR features is even poorer due to other frequent lung infections or HIV-associated diseases (128). There is relatively scarce data about CXR features in confirmed tuberculosis cases in HIV-infected children.

A list of suggested CXR features consistent with tuberculosis and a review process methodology have been proposed for diagnostic studies of childhood tuberculosis (179). To the best of our knowledge, there has been no publication on the use of this review process and the value of these radiographic signs for diagnosis of tuberculosis in HIV-infected children.

5.2 Berteloot, Marcy *et al* - Submitted

This radiological sub-study is implemented in 403 children enrolled in the PAANTHER 01 cohort with all CXR reviews available, excluding those who were enrolled only on the basis of CXR anomalies only. The results of this study have been presented at the congress of the Francophone Society for Pediatric and Prenatal Imagery (Société Francophone d'Imagerie Pédiatrique et Périnatale) in Liège, Belgium, on September 22 and 23, 2017, and have subsequently been submitted for publication with the following manuscript.

Value of Chest X Ray in the diagnosis of tuberculosis in HIV-infected children living in resource limited countries. Results from the ANRS 12229 - PAANTHER 01 study

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ABSTRACT

Objective: In this study we aimed to evaluate inter-reader reproducibility and diagnostic accuracy of standard chest radiography (CXR) in the diagnosis of tuberculosis (TB) in HIV-infected children.

Methods: Prospective study conducted in Burkina Faso, Cambodia, Cameroon, and Vietnam from April 2010 to May 2014. HIV-infected children aged ≤ 13 years with a clinical suspicion of tuberculosis were enrolled. Three readers including local radiologists (LR), a pediatric pulmonologist (PP), and a pediatric radiologist (PR) independently reviewed every CXR, blinded to any other conditions. Agreement was assessed on the presence and location of lesions consistent with TB: alveolar opacity, excavation, ghon focus, miliary, nodular infiltrates, enlarged paratracheal or perihilar lymph nodes, tracheal or bronchial compression, pleural effusion, gibbus. We assessed diagnostic accuracy of CXR after final consensus in a case/control analysis (culture- confirmed TB / unlikely TB, alive without TB treatment at 6 months).

Results: 403 children (median age 7.3 years [IQR: 3.5-9.7], 49.6% males) were enrolled in the study. Inter-reader agreement on lesions consistent with TB, expressed by Kappa coefficient were as follows: LR versus PP: $K=0,36$ [95%CI 0,27-0,45]; LR vs PR 0,16 [0,08-0,24]; PP vs PR 0,30 [0,21-0,40]. Among 51 cases and 151 controls, CXR after final consensus had a sensitivity of 71.4% [58.8-84.1] and a specificity of 50.0% [41.9-58.1] and an overall diagnostic accuracy of 55.3%.

Conclusion: Analysis of CXR showed poor to fair inter-reader agreement and limited diagnostic accuracy for TB in HIV-infected children, probably because of the frequency of co-morbidities, while accurate and rapid diagnosis is needed to reduce TB-related mortality. Radiological criteria should be discussed for this specific population.

INTRODUCTION

In children, tuberculosis (TB)-related mortality represents 239,000 deaths per year worldwide, more of 70% occurring in the south-east Asian and African regions, and 40,000 deaths occurring in HIV-infected children(1). Accurate and rapid diagnosis of tuberculosis is of major concern for a timely access to treatment and to reduce mortality (2), particularly in resource-limited countries with high TB and HIV burden. Inter-relation between Mycobacterium Tuberculosis (MTB) and HIV infections have been highlighted with an higher incidence of TB among HIV-infected children (3) who present an even poorer prognosis for Tuberculosis (TB) (4) and because MTB may induce acceleration of HIV progression(5).

In children, microbiological confirmation of pulmonary TB is difficult to obtain, ranging from 15% to 66% of cases (6,7). Hence, diagnosis of pulmonary TB in children is often based on the association between non-specific symptoms, history of exposure to a TB case, and CXR anomalies, particularly in low-resource countries where no alternative, more reliable, imaging methods such as computed tomography are available(8). Therefore, the presence of CXR anomalies consistent with TB is one of the major arguments for the initiation of treatment in children (6,9).

However, there is often a lack of physicians with adequate knowledge on tuberculosis CXR features in children in local hospitals from low-resource settings. Teleradiology programs have been developed but their implementation is difficult and the performance of training programs is still controversial(10–12). Furthermore, CXR images of TB disease in children are often non-specific (13), with limited diagnostic accuracy and inter-reader agreement(14). To take into account both the difficulty of reading paediatric CXR and the diagnostic importance of this exam, a standardized reading process with predefined terms has been proposed as part of the consensus case definitions of childhood TB for research(15). Such standardized process had been reported to be efficient in TB screening in HIV-infected adults(16).

Moreover, CXR features of TB are probably less specific in HIV-infected children because of other co-infections or HIV-associated diseases such as acute chest infections, aspiration, cardiovascular diseases, lymphocytic interstitial pneumonitis or pulmonary neoplasia(17). To the best of our knowledge, CXR has been poorly analyzed in prospective series of culture-confirmed TB in HIV-infected children(18–24). In particular, these series did not use a standardized form and did not take into account the degree of HIV-related immunodeficiency in radiological interpretation. None of these studies included a radiologist among the authors(17).

We aimed to evaluate inter-reader agreement on CXR in HIV-infected children with a clinical suspicion of TB between local readers and readers from a European center, with high experience in pediatric thoracic imaging, using a standardized form for reading and reporting anomalies (25). We

also aimed to assess diagnostic accuracy of CXR for pulmonary TB in this population and in a sub-group of HIV-infected children with severe immunodeficiency.

METHODS

This study was based on the ANRS 12229 PAANTHER 01 cohort, which was conducted in 8 hospitals in Burkina Faso, Cambodia, Cameroon, and Vietnam from April 2011 to December 2014. The study design has been previously described (7). HIV-infected children aged ≤ 13 years, with a suspicion of tuberculosis based on at least one of the following: persistent cough, fever > 2 weeks, failure to thrive, failure of antibiotics for a pulmonary infection, suggestive CXR, were eligible, excluding those with anti-tuberculosis treatment started within 2 years before inclusion. For this radiological sub-study, we excluded children whose only inclusion criteria was a suggestive CXR and those who had either a CXR or review missing. The TB status was defined as confirmed TB, unconfirmed TB and TB-unlikely, using the updated version of the Intrathoracic Tuberculosis Definitions for Diagnostic Research in Children (6). Children were clinically followed-up for 6 months after enrolment.

The study was approved by relevant national ethics committees, institutional review boards, and national authorities. The ANRS 12229 PAANTHER 01 study is registered at ClinicalTrials.gov (identifier NCT01331811).

Anteroposterior (AP) and left lateral CXR views were concomitantly performed for children of age ≤ 5 years and postero-anterior views (PA) for children older than 5 years. In case of unacceptable quality of the AP or PA view, a new CXR was performed, only once for the sake of radioprotection.

Three readers independently reviewed all CXR images: local radiologists (LR) from participating sites, considered as one reader, a pediatric pulmonologist (PP), and a thoracic pediatric radiologist (PR), both from a European children's hospital with high experience in pediatric thoracic imaging and TB. Readers were blinded to clinical or biological conditions. After checking quality criteria and validating the CXR for interpretation, readers had to perform a systematic analysis of the CXR, using a standardized form specifically designed for the study and based on previous recommendations(26). Each reader had to report and localize elementary lesions or anomalies affecting lung parenchyma, lymph nodes, airways, pleura, heart and bones (27).

Statistical analysis

For each reader, the suspicion of TB was based on the presence on CXR of at least one lesion among the following: Ghon focus, alveolar opacities, miliary pattern, nodular opacities, excavation, enlarged para-tracheal and/or peri-hilar lymph nodes, tracheal compression, bronchial compression, pleural

effusion, and gibbus. We assessed inter-reader agreement two by two, using Cohen's kappa coefficient (K)(28).

To assess CXR features, we performed a two-round process of final consensus as suggested by Graham *et al* (15). The primary readers were the LR and the PP. CXR were considered consistent with tuberculosis if primary readers agreed on the presence and location (right/left) of at least one of the lesion cited above as consistent with TB. The PR's blinded review settled discordant opinions between the LR and the PP on the presence and location of lesions consistent with TB. A final consensus could not be reached if the three readers disagreed on the presence and location of lesions. The final consensus on CXR anomalies was confronted to the TB status of children as bacteriologically confirmed or unconfirmed TB versus TB unlikely.

As CXR significantly contributes to the final diagnosis in children which are not bacteriologically confirmed (6), we assessed diagnostic accuracy of CXR in a nested case/control sub-analysis, considering as cases children with culture and/or Xpert MTB/RIF confirmed TB, and controls, children which were alive at 6 months without any anti-TB treatment. We assessed sensitivity and specificity of the CXR consistency with TB, and of elementary lesions as determined by the individual readers and by the final consensus process, for the diagnosis of TB and determined their 95% confidence intervals (95%CI). Overall diagnostic accuracy (ODA), defined by the proportion of correctly classified children(29), and p-value were used to compare performances of each reader and of the final consensus in the correct diagnosis of TB. We also assessed diagnostic accuracy of the final consensus on CXR and elementary lesions in the sub-group of children with severe immunodeficiency as defined by the WHO Immunological Classification (30).

Data are presented as median values and interquartile range (IQR). We performed all analyses using the Stata software version 13.0 (STATA Corp., Texas, USA) and the SAS software version 9.3 (SAS Institute, Cary, NC, USA). For all tests, a p-value <0.05 was considered as statistically significant.

RESULTS

Of 438 children initially enrolled in the PAANTHER 01 study, 14 had no CXR performed, 6 had missing CXR reviews because of unacceptable quality of CXR, and 15 children had been initially enrolled on the exclusive basis of a CXR anomaly; 403 children were finally included in this radiological study. Their median age was 7.25 years [IQR: 3.53-9.72], 200 (49.6%) were males, 63 (15.6%) were from Burkina-Faso, 130 (32.2%) from Cambodia, 108 (26.8%) from Cameroon, and 102 (25.3%) from Vietnam. Their median weight-for-age Z score was -2.5 [IQR: -3.3 to -1.8], and median CD4 percentage was 13.5% [IQR: 3.0 – 24.0]. Inclusion criteria were: persistent cough in 352 (87.3%), fever >2 weeks in 220 (54.6%), failure of antibiotics for a pulmonary infection in 180

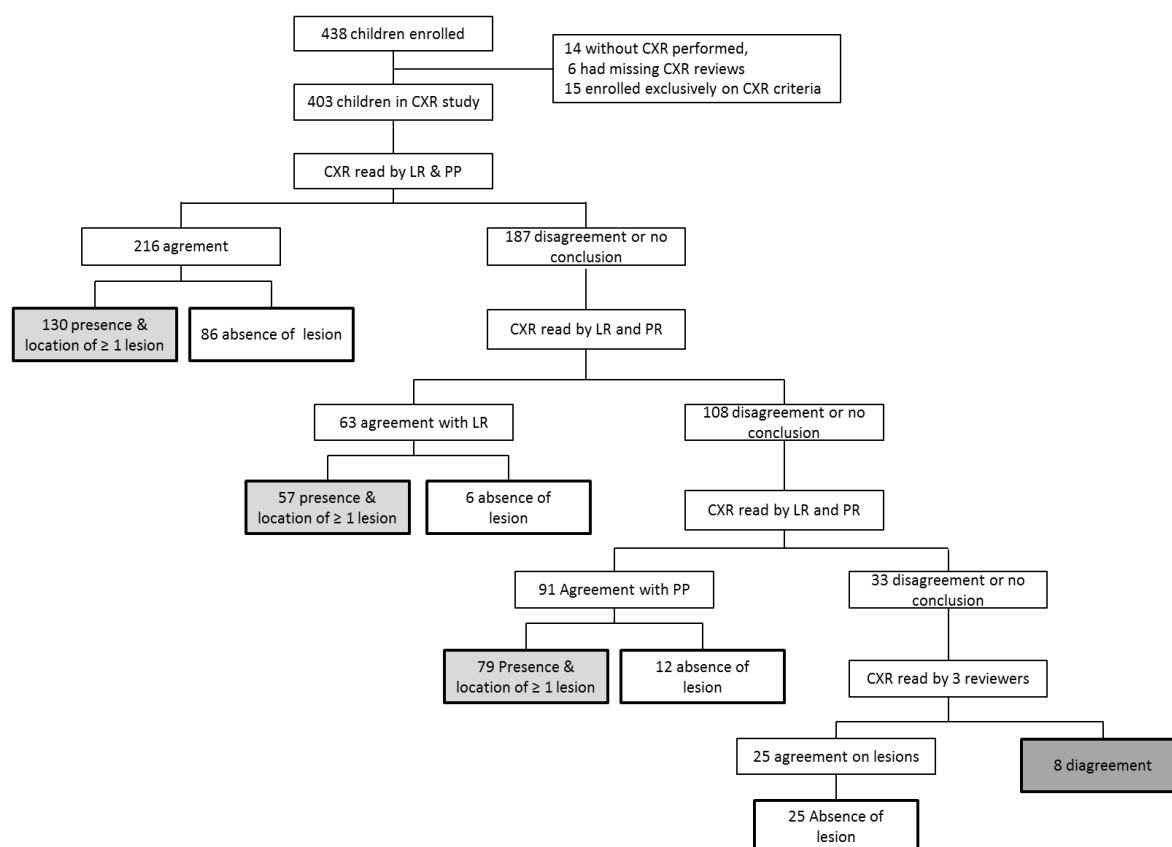
(44.7%), suggestive CXR anomaly in 149 (37%), and failure to thrive in 168 (41.7%). Applying the Graham 2015 classification, 51 (12.7%) children were retrospectively classified as confirmed TB (41 confirmed by culture and 10 by Xpert MTB/RIF only), 183 (45.4%) as unconfirmed TB, and 169 (41.9%) as unlikely TB. After 6 months of follow-up, 151 children still in follow-up had not received any anti-TB treatment and were considered as the control group.

Chest X-ray quality as assessed as good in 124 (30.8%), acceptable medium quality in 226 (56.1%), and not acceptable in 48 (11.2%) by the PP; information was missing in 5 (1.2%). It was assessed as good in 38 (9.4%), acceptable medium quality in 341 (84.6%), and not acceptable in 12 (3.0%) by the PR; information was missing in 12 (3.0%).

Inter-reader and final consensus on the cohort:

Kappa coefficients for two by two inter-reader agreement on absence or CXR consistency with TB and presence and location of elementary lesions are presented in table 1. Inter-reader agreement was poor to fair. The best inter-reader agreement on CXR consistency with TB was found between the LR and PP, probably mainly based on their agreement for presence and location of alveolar opacities while the PP and PR had a better agreement on elementary lesions such as ghon focus, alveolar opacities, miliary and nodular opacities. Inter-reader agreement was particularly poor on the presence and location of perihilar and paratracheal enlarged lymph nodes.

The two-round agreement process is detailed on figure 1. It led to a final consensus on the absence of lesion consistent with TB in 131 (32.5%) CXR and on the presence and location of at least one lesion consistent with TB in 264 (65.5%); a final consensus could not be reached in 8 (2.0%) CXR. The frequency of elementary lesions as determined after final consensus in the entire cohort is detailed in table 2, according to the TB status. The lesions most frequently detected and statistically associated with confirmed and unconfirmed cases of TB were alveolar opacities ($p<0.0001$), enlarged peri-hilar or paratracheal lymph nodes ($p<0.0001$), nodular opacities ($p=0.023$), and miliary pattern ($p=0.0489$). Bronchial compression was less frequent but statistically associated with the TB status ($p=0.0403$).



Diagnosis accuracy of CXR in the case control sub-study

Diagnostic accuracy of each single reader is presented in table 3 and additional contents. The LR had lower sensitivity but higher specificity as compared to both the PP and PR. Overall diagnostic accuracy was limited for the PR despite very high sensitivity, because of the high proportion of lesions detected in both cases and controls (94% and 80% respectively).

Although the PP and PR identified lesions in $\geq 75\%$ of CXR (table 3), and because of the relatively low sensitivity of the LR, after the final agreement process, 14 CXR (28.6%) of patients with confirmed TB were considered as normal.

CXR after final consensus had a sensitivity of 71.4% and specificity of 50% (overall diagnostic accuracy of 55.3%) (Table 4). Most of the elementary lesions could be detected in both cases and controls. Lesions which had the best sensitivity were alveolar opacities and the presence of enlarged paratracheal and/or peri-hilar lymph nodes (42% and 40% respectively). As these were also detected in controls, they were not significantly discriminative ($p=0.3978$ and $p=0.2808$). Miliary and/or nodular opacities pattern were less frequently detected (sensitivity of 25%) but had a high specificity (94.3%) and a significant p -value ($p=0.037$ and $p=0.0155$, respectively). All in whole, after final consensus on CXR, there was a statistically significant difference between cases and controls on the presence of at least one lesion consistent with TB ($p=0.0089$).

When restricting the analysis to children with severe immunodeficiency, the frequency of detected lesions increased in both cases and controls, leading to a lower specificity and overall diagnostic accuracy of CXR for TB (table 5).

DISCUSSION

In our study, we found a poor to fair inter-reader agreement on the detection of CXR elementary lesions in a cohort of 403 HIV-infected children presenting a clinical suspicion of TB. A majority of children presented lesions consistent with TB while nearly a third of patients with confirmed TB had no lesion detected on the CXR after a final-consensus process. Alveolar opacities and enlarged mediastinal lymph nodes were frequently found but had limited specificity for TB, especially in children with severe immunodeficiency. Conversely, miliary or nodular opacities patterns were less frequent and more specific for TB. Globally, sensitivity and sensibility of CXR for TB were 71.4 and 50% respectively.

Our inter-reader agreement results on CXR were poorer than previously reported (14,31,32), either for standardized reporting process of various chest radiographic anomalies in HIV-infected children, where inter-rater agreement was reported from moderate to high (31), or for the detection of mediastinal lymphadenopathies by CXR in children with suspected TB (14). However, none of these previous results integrated the location of lesions which is an additional factor of disagreement but probably better reflects the reality of lesions(33).

CXR detected more than 2/3 of children with culture-confirmed tuberculosis in our study with a specificity of 71%. Sensitivity reached 82% in children with severe immunodeficiency but specificity in this group was poorer (36%). In a recent study, Seddon *et al* (11) found a sensitivity (0.67) and specificity (0.48) of CXR alone for the diagnosis of childhood TB, which is consistent with our results. On a cohort of one hundred children with suspected TB, when confronting CXR to computed tomography, Swingler *et al*(14) found better sensitivity (67%) and specificity (59%) of CXR in detecting mediastinal lymphadenopathy than we did.

Elementary lesions that were reported after our agreement process were different from previous studies. We found a high specificity for TB of large airways compressions but it was rarely reported while it was previously reported in 28.7% of cases in a cohort of 188 infants and children with proven TB, with elevated diagnostic accuracy for TB as compared to other lower respiratory tract infections (33). This discrepancy could be explained by an older median age in our cohort (7.25 years vs 25.9 months) as airway compression occurs in TB with higher frequency in infants(34). In a prospective study describing radiological features in 126 HIV-infected children with culture-confirmed TB (22), Schaaf *et al* described frequency and types of lesions quite different from our results:

lymphadenopathies (56.3% of cases vs 40% of our cases), large airway compression (13.5% vs 2%), alveolar opacities (66.7% vs 42%), cavitation (26.2% vs 3.9%), pleural effusion (13.5% vs 5.9%), miliary (15.1% vs 11.8%), ghon focus (2.4% vs none), normal CXR (4.0% vs 27.5%). However, the authors themselves drew attention on a possible selection bias of sicker children in this hospital-based study and their results were not compared to a cohort of HIV-infected children without TB. Moreover, the results of this study in which all CXR were read by a single TB specialist are hardly comparable to our final consensus process between 3 reviewers; they are closer instead to the results obtained by the only reading of our pediatric thoracic radiologist.

The association of a clinical suspicion of TB and CXR anomalies is one of the major argument, according to experts' recommendations for diagnosis of bacteriologically unconfirmed TB in children(15). We found that alveolar opacities, peri-hilar or paratracheal enlarged lymph nodes, nodular opacities, and miliary pattern were significantly associated with a diagnosis of confirmed or unconfirmed TB in our cohort. Indeed, at the time of inclusion in the study, the presence these CXR anomalies was probably one of the major arguments to consider that these children had TB in the absence of bacteriological confirmation. However, in the case-control sub-study, we found that alveolar opacities and enlarged lymph nodes did not discriminate significantly cases from controls.

Our results also suggest that, in HIV-infected children, increasing the detection of lesions leads to decreasing specificity for TB. Indeed, the sensitivity of the readers increased with their experience in pediatric thoracic imaging but the overall diagnostic accuracy of the pediatric thoracic radiologist was impaired by a higher detection of lesions in both cases and controls while, in a previous study, pediatric radiologists demonstrated higher sensitivity and specificity for TB in children as compared to non-radiologists clinicians (12). Moreover, specificity of CXR for TB after final consensus was lower in severely immunocompromised children as, in this population, we found a higher frequency of CXR lesions in both children with and without TB disease. Indeed, HIV-infected children may present a wide range of possible co-morbidities and co-infections affecting CXR with similar features to those of TB (17). In 2001, Norton *et al* (35) found a frequency of chronic and transient lung changes associated with various etiologies on CXR of 40.3% and 31.8%, respectively, in a cohort of 201 HIV-infected infants and children; chronic lung changes were associated with lower CD4 cell counts. This notion does not call into question the need to improve the ability of local readers to detect radiological lesions(11,12,36), which is critical to reduce false negatives, as shown in our study, and therefore to reduce TB-related mortality and morbidity by enabling TB treatment. However, the high frequency of false positive CXR readings in our study may also hinder appropriate treatment of other HIV-related pulmonary affections.

Our results therefore highlight challenges in using CXR for the diagnosis of microbiologically-negative TB in HIV-infected children, and suggest the need to reconsider the use of CXR features in diagnostic classification systems for reference standards in diagnostic studies of HIV-infected children (36).

There are several limitations to our study, including the poor to fair inter-reader agreement itself, the heterogeneity of CXR quality, the lack of information on potential differential diagnosis for CXR anomalies due to the initial design of the study, and the possible bias in selecting controls as most patients with unconfirmed-TB received anti-TB treatment, excluding other possible false CXR-positive patients from the control group. However, to the best of our knowledge, this is the first multi-center and multi-country prospective study on CXR anomalies with a multiple reading process including local readers and a pediatric thoracic radiologist and analyzing the results in the light of the level of immunodeficiency. We thus believe that our results are applicable to HIV-infected children with clinical suspicion of TB in low-resource countries.

Conclusion

Analysis of CXR had poor to fair inter-reader reproducibility and limited diagnostic accuracy for TB in HIV-infected children, especially in those with severe immunodeficiency, probably because of the frequency of co-morbidities and co-infections. Miliary and nodular patterns correctly oriented on TB diagnosis while other lesions, usually considered as consistent with TB, could be misleading in HIV-infected children. Adapted radiological criteria and relevance of CXR in the diagnosis of unconfirmed TB should be discussed for this specific population.

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Table 1: Two-by-two inter-reader agreement on the presence of lesions consistent with tuberculosis on the chest radiograph (Cohen's Kappa)

	LR/PP	LR/PR	PP/PR
	K [CI95%]	K [CI95%]	K [CI95%]
TB suspicion on CXR	0,36 [0,27-0,45]	0,16 [0,08-0,24]	0,30 [0,21-0,40]
Ghon Focus	0,16 [-0,01-0,33]	0,12 [-0,02-0,28]	0,39[0,21-0,57]
Alveolar Opacities	0,34 [0,27-0,41]	0,20 [0,14-0,25]	0,39 [0,32-0,45]
Miliary	0,16 [-0,01-0,33]	0,13 [-0,02-0,28]	0,39 [0,22-0,57]
Nodular Opacities	-0,04 [-0,09 to 0,01]	0,10 [0,03 to 0,16]	0,19 [0,10 to 0,27]
Paratracheal Nodes	0,02 [-0,04-0,10]	-0,01 [-0,03 to 0,00]	0,10 [0,04 to 0,15]
Perhilar Nodes	0,04 [-0,03-0,12]	0,04 [-0,01-0,09]	0,12 [0,07-0,17]
Tracheal Comp	0,12 [-0,04 to 0,29]	-0,02 [-0,04 to -0,00]	-0,01 [-0,10 to 0,08]
Bronchial comp	0,06 [-0,04-0,16]	-0,04 [-0,07-0,00]	0,11 [0,03-0,19]
Excavation	-0,01 [-0,02 to -0,01]	0,02 [-0,01 to 0,04]	0,19 [0,03 to 0,36]
Pleural effusion	0,52 [0,31 - 0,72]	0,30 [0,15-0,45]	0,31 [0,16-0,45]
Gibbus	NA*	NA*	-0,003 [-0,008 to 0,001]

CXR: chest radiography; LR: local radiologist; PP: pediatric pulmonologist; PR: pediatric radiologist; TB: tuberculosis. *Kappa not measurable (all answers in one category for one reviewer)

Table 2: Association between CXR anomalies after final consensus and TB status

	Confirmed TB		Unconfirmed TB		Unlikely TB		P-Value (2 cat)
	N	(%)	N	(%)	N	(%)	
CXR consistent with TB							<.0001
Yes	35	(68.6)	152	(83.1)	77	(45.6)	
No	14	(27.5)	30	(16.4)	87	(51.5)	
Disagreement	2	(3.9)	1	(0.5)	5	(3.0)	
Ghon Focus							1.0000
Presence ¹	0	(0.0)	1	(0.5)	1	(0.6)	
Absence ²	51	(100.0)	182	(99.5)	167	(98.8)	
Disagreement ³	0	(0.0)	0	(0.0)	1	(0.6)	
Alveolar Opacities							<.0001
Presence	21	(41.2)	109	(59.6)	56	(33.1)	
Absence	29	(56.9)	74	(40.4)	110	(65.1)	
Disagreement	1	(2.0)	0	(0.0)	3	(1.8)	
Miliary							0.0489
Presence	6	(11.8)	5	(2.7)	2	(1.2)	
Absence	45	(88.2)	177	(96.7)	166	(98.2)	
Disagreement	0	(0.0)	1	(0.5)	1	(0.6)	
Nodular Opacities							0.0023
Presence	9	(17.6)	26	(14.2)	9	(5.3)	
Absence	42	(82.4)	156	(85.2)	159	(94.1)	
Disagreement	0	(0.0)	1	(0.5)	1	(0.6)	
Excavation							0.1417
Presence	2	(3.9)	5	(2.7)	1	(0.6)	
Absence	49	(96.1)	177	(96.7)	167	(98.8)	
Disagreement	0	(0.0)	1	(0.5)	1	(0.6)	
Paratracheal Enlarged Nodes							<.0001
Presence	3	(5.9)	13	(7.1)	6	(3.6)	
Absence	48	(94.1)	165	(90.2)	162	(95.9)	
Disagreement	0	(0.0)	5	(2.7)	1	(0.6)	
Peri-hilar Enlarged Nodes							0.5120
Presence	19	(37.3)	100	(54.6)	46	(27.2)	
Absence	31	(60.8)	82	(44.8)	122	(72.2)	
Disagreement	1	(2.0)	1	(0.5)	1	(0.6)	

Tracheal Compression							0.0403
Presence	1	(2.0)	1	(0.5)	0	(0.0)	
Absence	50	(98.0)	180	(98.4)	167	(98.8)	
Disagreement	0	(0.0)	2	(1.1)	2	(1.2)	
Bronchial Compression							0.1465
Presence	0	(0.0)	14	(7.7)	3	(1.8)	
Absence	51	(100.0)	169	(92.3)	164	(97.0)	
Disagreement	0	(0.0)	0	(0.0)	2	(1.2)	
Pleural effusion							0.7225
Presence	3	(5.9)	7	(3.8)	6	(3.6)	
Absence	48	(94.1)	176	(96.2)	162	(95.9)	
Disagreement	0	(0.0)	0	(0.0)	1	(0.6)	
Gibbus							NA
Presence	0	(0.0)	0	(0.0)	0	(0.0)	
Absence	51	(100.0)	183	(100.0)	168	(99.4)	
Disagreement	0	(0.0)	0	(0.0)	1	(0.6)	

Consensus on the presence and location of lesions¹, or consensus on absence of lesion² or absence of agreement between the three readers³. CXR: chest radiography; TB: tuberculosis.

Table 3: Diagnostic accuracy of each reader on the case control study

	Sensitivity*			Specificity*			ODA	p value
	n/N	%	(95%CI)	n/N	%	95%CI		
Local Radiologist	31/51	60.8	(47.4 - 74.2)	85/149	57.0	(49.1 - 65.0)	58.0	0.0277
Pediatric Pulmonologist	38/51	74.5	(62.5 - 86.5)	72/150	48.0	(40.0 - 56.0)	54.7	0.0049
Pediatric Thoracic Radiologist	47/50	94.0	(87.4 - 100.0)	28/143	19.6	(13.1 - 26.1)	38.9	0.0244

* excluding missing values

Table 4: Diagnostic accuracy of CXR features as determined by final consensus; case/control sub-study

	Sensitivity*			Specificity*			Overall Diagnostic accuracy	p-value
	n/N	%	(95%CI)	n/N	%	(95%CI)	%	
CXR consistent with TB	35/49	71.4	(58.8 - 84.1)	74/148	50.0	(41.9 - 58.1)	55.3	0.0089
Agreement on presence and location* of								
Ghon focus	0/51	0.0	(0.0 - 7.0)	150/151	99.3	(98.0 - 100.0)	74.3	1.0000
Alveolar opacities	21/50	42.0	(28.3 - 55.7)	97/150	64.7	(57.0 - 72.3)	59.0	0.3978
Miliary	6/51	11.8	(2.9 - 20.6)	149/151	98.7	(96.9 - 100.0)	76.7	0.0037
Nodular opacities	9/51	17.6	(7.2 - 28.1)	143/151	94.7	(91.1 - 98.3)	75.2	0.0155
Excavation	2/51	3.9	(0.0 - 9.2)	150/151	99.3	(98.0 - 100.0)	75.2	0.1576
Para-tracheal lymph nodes	3/51	5.9	(0.0 - 12.3)	145/151	96.0	(92.9 - 99.1)	73.3	0.6947
Peri-hilar lymph nodes	19/50	38.0	(24.5 - 51.5)	106/151	70.2	(62.9 - 77.5)	62.2	0.2808

Tracheal compression	1/51	2.0	(0.0 - 5.8)	150/150	100.0	(97.6 - 100.0)	75.1	0.2537
Bronchial compression	0/51	0.0	(0.0 - 7.0)	147/150	98.0	(95.8 - 100.0)	73.1	0.5725
Pleural effusion	3/51	5.9	(0.0 - 12.3)	145/151	96.0	(92.9 - 99.1)	73.3	0.6947
Gibbus	0/51	0.0	(0.0 - 7.0)	151/151	100.0	(97.6 - 100.0)	74.8	NA
Miliary and/or nodular opacities	13/51	25.5	(13.5 – 37.5)	148/157	94.3	(90.6 – 97.9)	76.0	0.0001
Bronchial and/or tracheal compression	1/51	2.0	(0.0 – 5.8)	153/156	98.1	(95.9 – 100.0)	70.0	1.0000
Peri-hilar and/or tracheal lymph nodes	20/50	40.0	(26.4 – 53.6)	110/157	70.1	(62.9 – 77.2)	62.8	0.0001

*excluding missing values. CXR: chest radiography; TB: tuberculosis.

Table 5: Diagnostic accuracy of CXR features as determined by final consensus; case/control sub-study, restricted to children with severe immunodeficiency

	Sensitivity*			Specificity*			Overall Diagnostic accuracy	P-Value
	n/N	%	(95%CI)	n/N	%	(95%CI)	%	
CXR consistent with tuberculosis	28/34	82.4	(69.5 - 95.2)	27/74	36.5	(25.5 - 47.5)	50.9	0.0484
Agreement on presence and location* of								
Ghon focus	0/35	0.0	(0.0 - 0.1)	76/76	100.0	(95.3 - 100.0)	68.5	NA
Alveolar opacities	17/34	50.0	(33.2 - 66.8)	38/75	50.7	(39.4 - 62.0)	50.5	0.9486
Miliary	5/35	14.3	(2.7 - 25.9)	74/76	97.4	(93.8 - 101.0)	71.2	0.0311
Nodular opacities	5/35	14.3	(2.7 - 25.9)	69/76	90.8	(84.3 - 97.3)	66.7	0.5132
Excavation	1/35	2.9	(0.0 - 8.4)	76/76	100.0	(95.3 - 100.0)	69.4	0.3153
Para-tracheal lymph nodes	3/35	8.6	(0.0 - 17.8)	73/76	96.1	(91.7 - 100.4)	68.5	0.3773
Peri-hilar lymph nodes	15/34	44.1	(27.4 - 60.8)	47/76	61.8	(50.9 - 72.8)	56.4	0.5554
Tracheal compression	1/35	2.9	(0.0 - 8.4)	75/75	100.0	(100.0 - 100.0)	69.1	0.3182
Bronchial compression	0/35	0.0	(0.0 - 0.1)	72/75	96.0	(91.6 - 100.4)	65.5	0.5500
Pleural effusion	3/35	8.6	(0.0 - 17.8)	71/76	93.4	(87.8 - 99.0)	66.7	0.7054
Gibbus	0/35	0.0	(0.0 - 0.1)	76/76	100.0	(95.3 - 100.0)	68.5	NA
Miliary and/or nodular opacities	9/35	25.7	(11.2 - 40.2)	70/78	89.7	(83.0 - 96.5)	69.9	0.0390
Bronchial and/or tracheal compression	1/35	2.9	(0.0 - 8.4)	74/77	96.1	(91.8 - 100.0)	67.0	1.0000
Peri-hilar and/or tracheal lymph nodes	16/34	47.1	(30.3 - 63.8)	48/78	61.5	(50.7 - 72.3)	57.1	0.4561

*excluding missing values

6 A diagnostic prediction score and algorithm for tuberculosis treatment decision in HIV-infected children with a suspicion of tuberculosis.

6.1 Research question and background

Our research on mortality and its determinant highlighted the need to introduce ART treatment early in HIV-infected children with a suspicion of tuberculosis, as well as the importance of prompt tuberculosis treatment initiation in children with confirmed and unconfirmed tuberculosis. Optimized algorithms are therefore needed for empirical tuberculosis treatment decision in those without smear-positive or Xpert-confirmed tuberculosis to reduce mortality and to accelerate initiation of ART.

Diagnostic scores have been used widely in all fields of medicine, from acute appendicitis to pulmonary embolism (Wells score), to leg symptoms in chronic vein disorders. They are designed to standardize and help medical decision making, often in the absence of key complementary exams. The Keith Edwards score has appeared in WHO guidance for diagnosis of tuberculosis in HIV-infected children which present significantly greater diagnostic challenges (139). It has not been incorporated in later pediatric guidance by the WHO (7, 8) as it was felt that existing diagnostic systems were too heterogeneous, lacked proper validation, and that WHO guidance by definition needed to be relevant to a large variety of epidemiological settings with variable resources and diagnostic capabilities (248). Other scoring systems or diagnostic approaches have been recommended by some national tuberculosis program, including the Kenneth Jones' criteria, also known as the Stegen-Toledo score which widely used in Latin America (170, 244)

Actual recommendations from the WHO contain no scoring system or diagnostic algorithm and do not provide weighting for clinical findings (8). However, a diagnostic algorithm or score would be very helpful for tuberculosis treatment decision, particularly in HIV-infected children, which were not well taken into account by previous systems, developed prior to the epidemic.

Based in different epidemiological settings and in countries with different health systems, the PAANTHER network was a unique opportunity to design a tuberculosis diagnostic algorithm for HIV-infected children, which was the primary objective of the ANRS 12229 PAANTHER 01 study.

6.2 Marcy *et al* – Submitted

The results of this study have been presented at the International AIDS Society conference in Paris, France, July 2017, and have been subsequently submitted for submission with the following manuscript.

A supplementary appendix has been submitted with the manuscript and can be found in Appendix 6 of this thesis.

A diagnostic prediction score and algorithm for anti-tuberculosis treatment decision in HIV-infected children with a suspicion of tuberculosis.

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Short title: A tuberculosis diagnostic score for HIV-infected children

Keywords: HIV, children, tuberculosis, diagnosis; prediction models

Abstract:

Background: Diagnosis of tuberculosis should be improved in HIV-infected children to shorten time to initiation of tuberculosis treatment and antiretroviral treatment and reduce mortality. We developed diagnostic prediction scores for tuberculosis treatment decision in HIV-infected children with a suspicion of tuberculosis.

Methods: HIV-infected children with a suspicion of tuberculosis were enrolled in the ANRS 12229 PAANTHER 01 Study in Burkina Faso, Cambodia, Cameroon, and Vietnam. Children underwent clinical assessment, chest radiography (CXR), Quantiferon Gold In-Tube (QFT) assays, abdominal ultrasonography, respiratory and stool sample collection for microbiology including Xpert MTB/RIF (Xpert). We used logistic regression to develop 4 diagnostic models for tuberculosis (confirmed or unconfirmed Vs. unlikely): 1) including all predictors, 2) excluding QFT, 3) excluding ultrasonography, 4) excluding QFT and abdominal ultrasonography. We secondarily included microbiology results in the models. We built scoring systems based on the models with the best area under receiver operating characteristic curves (AUROC) and predicted probability cutoffs obtaining a sensitivity >90%.

Findings: 438 children were enrolled in the study with median age 7.3 years, 220 (50.2%) male, 172 (39.3%) on ART at inclusion. 251 (57.3%) children had tuberculosis (55 (12.6%) culture or Xpert-confirmed and 196 (44.7%) unconfirmed). The final 4 models developed in 335 children with all predictors available included: fever lasting >2 weeks, unremitting cough, hemoptysis and weight loss in past 4 weeks, contact with a smear-positive tuberculosis case, tachycardia, miliary, alveolar opacities, and lymph nodes on CXR, abdominal lymph nodes on ultrasonography, and QFT results. The AUROCs were 0.839, 0.830, 0.819, 0.808, and 0.866, 0.861, 0.850, 0.846 when including Xpert, for model 1, 2, 3, and 4, respectively. Scores developed on model 2 with and without Xpert had a sensitivity of 91.5% or 88.6% (with Xpert) and a specificity of 42.5% or 61.2% (with Xpert) for the diagnosis of tuberculosis.

Conclusions: The developed scores have a good diagnostic performance, especially if Xpert is

available. Used in an algorithm, they should enable to make prompt treatment decision in children with presumptive tuberculosis and a high mortality risk, thus contributing to a significant public health benefits.

INTRODUCTION

Diagnosis is a major challenge in childhood tuberculosis. In 2015, only 359,000 (36%) of the estimated one million tuberculosis cases occurring in children were notified to the World Health Organization (1). This low notification rate, partly explained by reporting issues in some countries, is mostly due to the lack of capacity to diagnose tuberculosis in children. This is explained by the low sensitivity of microbiological exams resulting from the paucibacillary nature of the disease and the difficulty to self-expectorate in children, the lack of a point-of-care test, and the limitations of clinical approaches (ref). Under-diagnosis leads to poor access to treatment and subsequent mortality (2). Recent mathematical modelling estimates show that of 239,000 annual pediatric tuberculosis deaths, more than 96% occurred in children not receiving anti-tuberculosis treatment (3).

Diagnostic challenges are greater in HIV-infected children (4). Microbiological diagnosis, including with the recently WHO-endorsed Xpert MTB/RIF (Xpert) seem to perform similarly in HIV-infected children than in HIV-negative children but complementary clinical and radiological diagnostic approaches lack specificity in the context of severe immune suppression and overlapping clinical features due to opportunistic infections and HIV-infection itself (4-6). Furthermore, immune deficiency also reduces sensitivity of immunological tests used to detect latent tuberculosis infection (7). As in HIV-negative children, poor access to anti-tuberculosis treatment is responsible for a large part of tuberculosis-related mortality in HIV-infected children. Of 40,000 tuberculosis-related deaths in HIV-infected children, representing more than 1/3 of pediatric HIV mortality, it is estimated that 90% of these occurred in children not receiving anti-tuberculosis treatment (3, 8).

Initiation of anti-tuberculosis treatment significantly reduces mortality in HIV-infected children with confirmed and unconfirmed tuberculosis, but may also lead to delayed antiretroviral therapy (ART); this is a serious issue as ART is associated with major reduction in mortality when started during the first month of follow-up (9). Use of Xpert on appropriate samples could lead to same-day treatment decision in these children most at risk of dying. In others, optimized algorithms or scoring systems for empirical anti-tuberculosis treatment decision are needed to help clinicians initiate anti-tuberculosis treatment appropriately and accelerate initiation of ART.

Various scoring systems and diagnostic approaches have been developed and used to help tuberculosis diagnosis in children (10, 11). However a comprehensive analysis of these approaches revealed their limited coherence, frequent absence of a standard definition of symptoms, poor performance in HIV-infected children, and inadequate validation (10-12). Although still in use in some countries, these scores are currently not recommended by the WHO (13, 14). A recent study however highlighted their potential interest in HIV-infected children (15, 16).

We aimed to build a diagnostic prediction score and algorithm for anti-tuberculosis treatment decision in HIV-infected children with a suspicion of tuberculosis, based on microbiological, clinical and radiological features. We assessed whether the Quantiferon Gold In-Tube (QFT), an Interferon Gamma Release Assay (IGRA) that can be used as a replacement test for tuberculin skin test (TST) for the diagnosis of tuberculosis infection, and abdominal ultrasonography, which has shown its interest for the diagnosis of tuberculosis in HIV-infected adults, had an added value in this score (17, 18).

METHODS

The ANRS 12229 PAANTHER 01 study was a diagnostic cohort study aiming at developing an algorithm to improve diagnosis of tuberculosis in HIV-infected children. It was conducted in 8 hospitals in Burkina Faso, Cambodia, Cameroon, and Vietnam from April 2011 to December 2014. The inclusion procedures and study design have been described elsewhere (19). In brief, we enrolled HIV-infected children aged ≤ 13 years with a suspicion of tuberculosis based on at least one of the following: 1) persistent cough and/or 2) fever > 2 weeks, 3) failure to thrive defined as recent deviation in the growth curve or a weight for age Z score $< -2SD$, 4) failure of antibiotics for a pulmonary infection, or 5) suggestive chest X-ray (CXR), and excluded those with anti-tuberculosis treatment started within 2 years before inclusion.

The study was approved by relevant national ethics committees, institutional review boards, and national authorities. The ANRS 12229 PAANTHER 01 study is registered at ClinicalTrials.gov (identifier NCT01331811).

Procedures and definitions

After parent or guardian informed consent, detailed history on presence and duration of

symptoms in the 4 weeks prior to enrolment was collected with a standardized questionnaire. Cough patterns were characterized using a graphic illustration shown to parents/guardian as acute cough with delayed recovery, recurrent acute cough, or persistent, non-remitting cough, as suggested before (20) (Supplementary appendix). All children underwent a complete physical exam, a chest radiograph (CXR), an abdominal ultrasonography, a TST, blood samples for HIV RNA, CD4, complete blood count, and transaminases measurements, as well as the QFT assay, and bacteriological specimen collection. Each child had 5 to 6 bacteriological samples collected over a period of 3 days for Xpert, smear microscopy, and mycobacterial culture. Based on the age of the child, sample collection included 2 to 3 gastric aspirates or expectorated sputa, as well as one nasopharyngeal aspirate, one stool sample, and one string test if aged ≥ 4 years, as detailed elsewhere, (19). ART and anti-tuberculosis treatment were initiated at the discretion of the treating physician. All children were followed in the study for 6 months, with visits at 1, 2, 3, and 6 months (M6) following inclusion. All data were collected using standardized paper case report forms and entered in an online database developed on the Voozanoo software (Epiconcept, Paris, France).

At the end of the study, children were retrospectively classified as confirmed tuberculosis, unconfirmed tuberculosis, or unlikely tuberculosis, using the updated version of the Intrathoracic Tuberculosis Definitions for Diagnostic Research in Children (Table S1, appendix) which was applied retrospectively (21). For the final prediction models, the reference diagnosis was tuberculosis defined as either confirmed or unconfirmed. CXRs were reviewed independently by two readers blinded to patient data; discordant opinions were resolved by a third reader. CXRs were considered consistent with tuberculosis if reviewers agreed on the presence and location of ≥ 1 lesion among the following: alveolar opacity, bronchial compression, excavation, ghon focus, gibbus, miliary, nodular infiltrates, paratracheal nodes, peri-hilar nodes, pleural effusion, and tracheal compression (22). The TST was considered positive if the transverse diameter of induration, measured at 48-72 hours with the ball-point pen technique, was >5 mm. The QFT result was interpreted according to the manufacturer's recommendation. It was not included in the reference standard. We used age-defined standards for tachycardia and tachypnea (Table S2, appendix) (23).

Sample size

The sample size was initially determined to allow random split-sample development and validation. With an expected sensitivity and specificity of 90% for the model, a precision of

15%, and expecting that 2/3 of children would be diagnosed with tuberculosis, we estimated that we would need ≥ 210 children in the development group and an identical number in the validation group (24). The study Scientific Committee secondarily approved the development of the model on the full dataset and internal validation using boot-strap resampling.

Statistical analysis

We described the characteristics of the study population and we compared baseline characteristics between groups using Student t-test or Kruskal-Wallis test and Pearson's chi-square or Fisher's exact test as appropriate.

We used logistic regression to develop diagnostic prediction models for tuberculosis. We handled missing data using complete-case analysis, restricting analysis to those children with data available for candidate predictors. We used two strategies to select predictors for the models. We included as candidate predictors characteristics used in previous childhood tuberculosis scoring systems and algorithms or characteristics known to be associated with the diagnosis of tuberculosis in children with HIV-infection according to the literature, and tests of specific interest for the PAANTHER study, such as the QFT test and abdominal ultrasonography results (see supplementary methods, supplementary appendix). To identify additional predictors, we also performed a nested diagnostic case-control study, selecting as cases children with culture-confirmed tuberculosis and as controls those with unlikely tuberculosis whom were still alive at M6 and had not been treated for tuberculosis. We included as predictors in the models CXR features as assessed by the local reader. We tested in the models various duration of symptoms (>2 , >3 , >4 weeks) and selected the one with the best Akaike Information Criterion (AIC).

We developed 4 different models: model 1 which integrated all predictors, model 2 which excluded QFT from candidate predictors, model 3 which excluded abdominal ultrasonography, and model 4 which excluded both QFT and abdominal ultrasonography, as these two exams may not be routinely available in many resource-limited settings. We selected predictors to keep in the final models using stepwise backward selection. As proposed for development of prediction models, we used a threshold of 0.135, corresponding to the AIC, when incorporating QFT results which had two degree of freedom (negative, positive, and indeterminate), or 0.157 when all variables had one degree of freedom (25). We assessed model calibration with the Hosmer-Lemeshow (HL) test and we estimated the

AUROC for each model obtained. We performed internal validation using bootstrap resampling (100 samples) to quantify optimism of the models; this enables to adjust the model AUROC to estimate its real performance. We did not include Xpert and smear microscopy results as initial predictors to avoid issues of separability in model development; we included them in final models using Firth's penalized likelihood.

We compared AUROCs of obtained models and selected the 2 best models, with and without microbiology, based on their discriminative ability. We applied the models to the case control study and identified predicted probability cutoffs reaching a sensitivity >90%. Using these cutoffs, we developed associated diagnostic scores by assigning each variable in the models a predictor score equal to the beta coefficient rounded to the nearest integer. We used McNemar's chi square test to compare sensitivity and specificity of scores with and without microbiology. We kept the score with the best diagnostic accuracy, based on sensitivity and specificity, and parsimony. We brought up the threshold to 100 to facilitate calculations and multiplied the predictors by the same multiplicative factor. This enabled to retain discrimination between betas, while keeping the count manageable.

We assessed diagnostic performance of the score obtained in the different countries, in children with or without very severe immunodeficiency defined as CD4 <10%, and in those with and without chronic cough as an inclusion criteria. We also assessed diagnostic performance of the score obtained in the overall cohort, considering missing data for predictors as all negative or all positive.

We performed all analyses using the SAS software version 9.3 (SAS Institute, Cary, NC, USA). We wrote this report following recommendations from the TRIPOD statement (26).

RESULTS

We enrolled 438 children in the PAANTHER 01 study. Their median age was 7.3 years and 220 (50.2%) were male. 172 (39.3%) were on ART at inclusion, and the median CD4+ cell percentage was 14.0. Tuberculosis was confirmed by culture and/or Xpert in 55 (12.6%) children. Additionally, 196 (44.7%) children were retrospectively classified as unconfirmed tuberculosis, for a total of 251 (57.3%) children with tuberculosis. Anti-tuberculosis treatment was initiated in 241 (55%) children; at a median time of 7 days after inclusion in the study. Other characteristics are presented in Table 1.

Performance of individual predictors

The individual predictors with the best sensitivity for the diagnosis of tuberculosis were a cough in the previous 4 weeks (94.0%), a cough lasting more than 2 weeks (84.8%), a cough in the past 24 hours (89.6%), a fever in the previous 4 weeks (83.6%), and weight loss in the previous 4 weeks (71.2%) (Table 2). Specificities of these signs were poor overall with values of 6.4%, 18.7%, 8.6%, 27.4%, and 43.3%, respectively.

Lymph nodes were identified on the ultrasonography in 108 (26.7%) of 405 children with the exam performed, with a sensitivity and specificity of 35.4% and 85.7%, respectively. CXR lesions with the best sensitivity were peri-hilar lymph nodes (36.8%) and alveolar opacity (32.7%), which had specificities of 88.0%, and 76.6%, respectively. QFT, which was available in 420 children, was positive in 52 (12.4%) and indeterminate in 98 (23.3%). Its sensitivity and specificity for the diagnosis of tuberculosis were 21.7% and 90.8% if indeterminate tests were not considered, and 16.0% and 92.6% if indeterminate tests were considered as negative.

Predictors associated with culture-confirmed tuberculosis in the case control sub-analyses

45 children with culture-confirmed tuberculosis were considered as cases and 153 children alive at M6 and not treated for tuberculosis were considered as controls. Their characteristics are presented in Table S3 (Supplementary appendix). Predictors that were associated with the diagnosis of tuberculosis in the case control sub-analysis were a fever lasting more than 2 weeks, hepatomegaly, tachycardia, abdominal lymph node and splenomegaly on ultrasonography, miliary features on the CXR, and the QFT results (Table 3). Tachycardia only was not part of our initial list of predictors.

Diagnostic prediction models

Of 438 children enrolled in the study, 335 had data available for all selected predictors and were included in the diagnostic prediction models, including 201 (60.0%) with tuberculosis and 134 (40.0%) classified as not-tuberculosis. Compared to children not enrolled in the model development, they were older, had higher WAZ and hemoglobin count, a higher ratio of NTM infection, and a higher proportion initiated on anti-tuberculosis treatment, and their risk of death was lower (Table S5, supplementary appendix).

An identical set of 9 predictors remained in the 4 final models: fever lasting more than 2 weeks, unremitting cough, hemoptysis in the previous 4 weeks, weight loss reported in the previous 4 weeks, contact with a smear-positive tuberculosis case, tachycardia, miliary, alveolar opacities, and lymph nodes on CXR (Supplementary appendix, Table S6). QFT results and abdominal lymph nodes on ultrasonography remained in final model 1; lymph nodes on ultrasonography remained in final model 2, which was developed excluding QFT, and QFT remained in final model 3 which was developed excluding ultrasonography from the initial predictors. When introduced as predictors in the 4 initial models, ART and CD4 percentage did not remain in final models. There was no interaction between predictors and ART or CD4 percentage.

The AUROCs for final models 1, 2, 3, and 4 were 0.839 (95%CI 0.797 - 0.880), 0.830 (95%CI 0.787 - 0.873), 0.819 (95%CI 0.775 - 0.863), 0.808 (95%CI 0.762 - 0.853), respectively. The AUROC of model 4 differed significantly from that of model 1 ($p=0.0191$) while the AUROC of model 2 and 3 were not statistically different ($p=0.220$ and $p=0.072$). All models showed good calibration (HL $p=0.475$; HL $p=0.518$; HL $p=0.990$; HL $p=0.742$). The optimism estimated by bootstrap resampling for models 1, 2, 3, and 4 was 0.0727 (95%CI: 0.0301 - 0.1154), 0.0705 (95%CI: 0.0244 - 0.1166), 0.0734 (95%CI: 0.0248 - 0.1219), and 0.0698 (95%CI: 0.0183 - 0.1212), respectively.

Performance of models integrating microbiological confirmation

When including bacteriological confirmation by Xpert on any sample as a predictor in the final models, the AUROC increased significantly to 0.866 (95%CI 0.829 - 0.904), 0.861 (95%CI 0.822 - 0.899), 0.850 (95%CI 0.809 - 0.890), and 0.846 (95%CI 0.805 - 0.887), for model 1, 2, 3, and 4, respectively, as compared to models without Xpert ($p=0.0015$, $p=0.0011$, $p=0.0007$, $p=0.0002$) (Figure 1). The AUROC of model 4 with Xpert differed significantly from that of model 1 with Xpert ($p=0.0499$) while the AUROC of model 2 and model 3 were not statistically different ($p=0.2800$ and $p=0.0799$, respectively). The optimism estimated by bootstrap resampling for models 1, 2, 3, and 4 with Xpert was 0.0621 (95%CI: 0.0180 - 0.0974), 0.0584 (95%CI: 0.0147 - 0.0872), 0.0623 (95%CI: 0.0225 - 0.1020), and 0.0533 (95%CI: 0.0069 - 0.0668), respectively.

Including smear microscopy on any sample as a predictor in the final models did not lead to significant changes for model 1, 2, and 3 (AUROC 0.846, 95%CI 0.805 - 0.886; 0.840, 95%CI 0.799 - 0.882; and 0.828, 95%CI 0.785 - 0.871), as compared to final models without

microbiology ($p=0.1725$, $p=0.1067$, $p=0.1112$). It led to a significant AUROC change for model 4 only (AUROC: 0.824; 95%CI 0.780 - 0.867), as compared to the final model without microbiology ($p=0.0281$). AUROCs of model 2, 3, and 4 did not differ significantly from that of model 1 ($p=0.2951$, $p=0.0825$, and $p=0.0517$).

Performance of the scoring system

Model 1 and model 2 with and without Xpert were selected for the score based on their discriminative ability. Applying these models to the case control population, the predicted probability cutoffs obtaining a sensitivity $>90\%$ were 0.36 and 0.37 for model 1 and model 1 with Xpert, 0.38 and 0.38 for model 2 and 2 with Xpert, with AUROCs of 0.8895, 0.9468, 0.8513 and 0.9340, respectively (Supplementary appendix figures S1). When using these cutoffs, the score developed on model 1 had the following diagnostic accuracy measures in the 335 children with predictors available: sensitivity 177/201, 88.1% (95%CI 83.6 – 92.5), specificity 75/134, 56.0% (95%CI 47.6 – 64.4), positive predictive value (PPV) 177/236, 75.0% (95%CI 69.5 – 80.5), negative predictive value (NPV) 75/99, 75.8% (95%CI 67.3 – 84.2). The score developed on model 1 with Xpert had the following diagnostic accuracy measures: sensitivity 179/201, 89.1% (95%CI 84.7 – 93.4), specificity 82/134, 61.2% (95%CI 52.9 – 69.4), PPV 179/231, 77.5% (72.1 – 82.9), NPV 82/104, 78.8% (95%CI 71.0 – 86.7). The score developed on model 2 had the following diagnostic accuracy measures: sensitivity 184/201, 91.5% (95%CI 87.7 – 95.4); specificity 57/134, 42.5% (95%CI 34.2 – 50.9); positive predictive value 70.5% (95%CI 65.0 – 76.0); negative predictive value 77.0% (95%CI 67.4 – 86.6). The diagnostic accuracy measures of the score developed using model 2 with Xpert were the following: sensitivity 178/201, 88.6% (95%CI 84.2 – 93.0); specificity 82/134, 61.2% (95%CI 52.9 – 69.4); positive predictive value 77.4% (95%CI 72.0 – 82.8); negative predictive value 78.1% (95%CI 70.2 – 86.0). Sensitivity did not differ between the scores with and without Xpert for built on model 1 and on model 2 ($p=0.4795$ and $p=0.0833$), while specificities differed significantly ($p=0.0082$, $p<0.0001$). There were no significant differences between sensitivities and specificities of the scores built on model 1 with Xpert and model 2 with Xpert ($p=0.7389$, $p=1.000$). Model 2 with Xpert is used for further analyses.

Diagnostic accuracy of the score in the different countries and groups

Globally, sensitivity of the score derived from model 2 with Xpert did not differ in the 4 countries with measures of 15/20 (75.0%), 92/100 (92.0%), 37/41 (90.2%), and 34/40 (85.0%), in Burkina Faso, Cambodia, Cameroon, and Vietnam, respectively ($p=0.144$).

Specificities however differed significantly with measures of 21/24 (87.5%), 13/30 (43.3%), 14/35 (40%), and 34/45 (75.6%), in Burkina Faso, Cambodia, Cameroon, and Vietnam, respectively ($p < 0.0001$). Sensitivity of the score did not differ between patients with CD4 $< 10\%$ (74/85, 87.1%) and those with higher CD4 count (104/116, 89.7%; $p = 0.568$), however, specificity was lower in those with CD4 $< 10\%$ (26/49, 53.1%) than those with higher CD4% (48/76, 63.2%, $p = 0.014$). Sensitivity of the score did not differ between children with chronic cough as an inclusion criteria and the others (150/169, 88.8% and 28/32, 87.5%; $p = 0.838$); neither did specificity (69/115, 60% and 13/19, 68.4%; $p = 0.485$).

When applying the score derived from model 2 with Xpert to the overall cohort, it identified correctly 228 (85.7%) children with tuberculosis and 116 (62.0%) children without tuberculosis, when considering all missing data as negative. Conversely, when considering all missing data for predictors as positive, it identified correctly 228 (90.8%) children with tuberculosis and 82 (43.9%) children without tuberculosis.

Proposed PAANTHER algorithm

The proposed PAANTHER algorithm is presented in figure 3. In HIV-infected children presenting with a clinical suspicion of tuberculosis, based either on chronic cough > 2 weeks or other symptoms or criteria including prolonged fever > 2 weeks, failure to thrive, failure of antibiotics for a respiratory infection, the PAANTHER score can be applied. It could also apply to children presenting a suggestive CXR, if it has been done as part of the initial diagnosis process. An anti-tuberculosis treatment should be initiated immediately in children with a score > 100 . A contact with a smear positive case is enough to score > 100 points and initiate anti-tuberculosis treatment. A CXR may not be needed for treatment decision in children reaching this threshold with history of disease and clinical assessment only. In children not reaching a score of 100 after full assessment including abdominal ultrasound exam, tuberculosis could be ruled out and these children could be followed up normally after subsequent clinical reassessment for persistent symptoms. If an abdominal ultrasound cannot be available permanently on site, an alternative score can be used (Supplementary appendix). Sensitivity is maintained at 181/201, 90.0% (95%CI 85.9 – 94.2), but specificity is lower at 65/134, 48.5 (95%CI 40.0 – 57.0).

DISCUSSION

We developed a diagnostic prediction score for anti-tuberculosis treatment decision in HIV-

infected children with a suspicion of tuberculosis enrolled in 4 countries. We aimed to provide clinicians from high-tuberculosis burden and low-income settings with a decision-making tool to initiate anti-tuberculosis treatment quickly in HIV-infected children with a suspicion of tuberculosis. The scoring systems obtained had a sensitivity of approximately 90% and specificities of 61% or 43% depending whether or not Xpert was included in the diagnostic strategy.

To our knowledge this is the first study developing a diagnostic score exclusively in HIV-infected children using methods recommended for diagnostic prediction models. Previous diagnostic scores and algorithms used for pediatric tuberculosis were mostly based on expert opinion and most often lacked validation (10, 11). A prospective study in South Africa with a similar approach showed that the combined presence of 3 symptoms at inclusion (unremitting persistent cough for more than 2 weeks, objective weight loss in the last 3 months, fatigue reported by the family) constitutes a good diagnostic approach of tuberculosis in South African non-HIV children aged ≥ 3 years (sensitivity 82%, specificity 90%) but that it performed poorly in those infected by HIV (sensitivity 56%, specificity 62%) (27). Recently a study applying retrospectively various scoring systems in HIV-infected children evaluated for tuberculosis in Brazil found that an extended version of this approach had a sensitivity of 94% or 84%, depending on whether or not microbiological evaluation was included in the evaluation, and a specificity of 30% (15). Other systems tested included a score used in Benin to evaluate BCG vaccination effectiveness in a global research program initiated by the WHO (28). This scoring system had a high specificity (93%) and a sensitivity of 83% when microbiological data was included; sensitivity however was only 45% when microbiology was not considered, which makes it less suitable for contexts where it might be lacking and for children without bacteriologic confirmation (15). Our proposed score has therefore good performances overall, as compared to these scoring systems, as it enables to reach a good sensitivity while maintaining an acceptable specificity if Xpert is available.

A number of predictors included in our final models have been used in previous scores, which were based on expert opinion (ref). This included prolonged fever >2 weeks, unremitting cough, reported weight loss, hemoptysis, contact with a smear-positive tuberculosis case. The vast majority of children enrolled in the study had prolonged cough as an inclusion criteria, which was therefore not specific. However unremitting cough, which was assessed by a seemingly more complex process using a graphic illustration rather than a simple question to

parents, showed a much higher specificity and remained associated to tuberculosis diagnosis in the model. Tachycardia, which was not used before in pediatric tuberculosis scores, is part of the 3 components used as danger sign which should trigger initiation of anti-tuberculosis treatment in severely-ill HIV-infected adults (29). It here contributes more to the overall score than reported weight loss in the past month which is less specific. Chest radiographs findings significantly contribute to our score, which may be challenging due to limited of access to quality CXR and lack of reading skills in limited-resource settings. We used the local reader's opinion which constitutes an imperfect test compared to more experienced readers but is more in line with the reality on the grounds (Berteloot et al. Submitted). Presence of lymph nodes on the ultrasonography had a similar diagnostic accuracy than found in HIV-infected Cambodian adults in whom it had a sensitivity of 32% and a specificity of 97% (17). Withdrawing this predictor from the model had a significant impact on the AUROC, hence its discriminative ability, which confirms its interest in this indication. Sensitivity of QFT was similar to that previously described in children with confirmed tuberculosis in a high HIV-burden setting and much lower than the sensitivity in HIV-infected children estimated to 47% by a recent meta-analysis (18, 30). It did not improve the model's discriminative ability, which confirmed its lack of interest for the diagnosis of tuberculosis in children with impaired immunity.

With an overall sensitivity around 90%, the scoring system could enable standardized and rapid treatment decision in HIV-infected children with presumptive tuberculosis. We showed previously that mortality was associated with the lack of anti-tuberculosis treatment rather than the delay to treatment initiation in ART-naïve children from the PAANTHER study (9). However initiation of anti-tuberculosis treatment led to delay ART, which is associated to significantly increased mortality. Prompt anti-tuberculosis treatment decision is therefore needed. It took a median of one week to initiate anti-tuberculosis treatment in the PAANTHER study. The developed score could help make a same day treatment decision in children presenting presumptive tuberculosis. The step-by-step algorithmic approach enables to make a decision without the use of CXR and abdominal ultrasound exam in children presenting clinical criteria. A recent cost-effectiveness analysis estimated that in high tuberculosis burden countries, it may be more cost-effective to treat all children with presumptive tuberculosis (31). In HIV-infected children however, pill burden and potential delays in ART initiation resulting from anti-tuberculosis treatment are calling for a more discriminant approach. In that sense, including Xpert in the model enables to maintain a

sensitivity of approximately 90% while improving its overall discriminative ability with a specificity of 61%. Diagnostic accuracy of this decision score is therefore poorer than the final clinical decision from clinicians following children in the study who treated 92% of children with tuberculosis and only 6% of those without; however, we expect that it will help make a faster treatment decision, especially when combined with ambulatory sample collection methods for Xpert, such as nasopharyngeal aspirates and stool samples, which have showed excellent feasibility and good sensitivity for culture-confirmed tuberculosis (19). In practice however access to treatment does not always depend exclusively on treatment decision and may be delayed for other structural reasons.

Our study has limitations. First, the most important limitation which is inherent to the question of childhood tuberculosis is the lack of a reference standard independent from predictors used in the model, unlike what has been done previously in HIV-infected adults where a microbiological reference standard is available (32). Arising from this lack of independence, incorporation bias may have led to overestimate the models' diagnostic performance (33). The good discriminative ability of the model in the case control subset however shows that this has limited impact on the performances of the score. Second, we used a reference standard which has not been validated for HIV-infected children and maybe subject to limitations, as highlighted before for the previous version of the classification system (21, 34, 35). Previous analyses however show that initiating anti-tuberculosis treatment is beneficial in both confirmed and unconfirmed tuberculosis cases (9). Third, almost $\frac{1}{4}$ of study participants had missing data for the considered predictors; these children were slightly younger than children from the development dataset and had a globally more severe clinical status which translated in a higher mortality rate. The sensitivity analysis shows however that the proposed score would have a stable sensitivity in these children, and that variation in missing data would mostly impact specificity which could vary between 43 and 61%. At last, eligibility criteria for our study differ from WHO's screening criteria to investigate for tuberculosis which are poor weight gain, fever, current cough, or history of contact with a tuberculosis case (36). This score is therefore not directly applicable to children presenting these criteria. Despite these limitations our study has strengths. Development over 4 countries with different diagnosis practices insures better external validity and generalizability of the scores. Secondly, we performed internal validation, an important component of model development, which shows that despite their assessed optimism, developed models would keep a good prediction performance in real life and other settings

(26).

Tuberculosis remains a major cause of death in HIV-infected children, accounting for more than 1/3 of mortality in this population. With its high sensitivity and algorithmic approach the score developed in the PAANTHER study should enable to initiate treatment rapidly in most children at risk of tuberculosis-related mortality in those presenting with presumptive tuberculosis. This treatment decision algorithm constitutes a consequentialist approach to tuberculosis in HIV-infected children, considering the need to initiate treatment to avoid lethal consequences of under-treatment, rather than an essentialist approach considering the trueness of the diagnosis of tuberculosis (37). The proposed algorithm has however not been fully tested and an external validation study is needed to validate and adapt both the scoring system and the overall approach, and evaluate its clinical usefulness.

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Table 1. Characteristics of children enrolled in the study

	All children		Not tuberculosis		Tuberculosis	
	n*	(N=438)	n*	(N=187)	n*	(N=251)
Age, years		7.3 (3.3 – 9.7)		7.3 (2.2 – 9.9)		7.3 (3.9 – 9.6)
Sex, male		220 (50.2)		82 (43.9)		138 (55.0)
Country						
Burkina Faso		63 (14.4)		35 (18.7)		28 (11.2)
Cambodia		139 (31.7)		36 (19.2)		103 (41.0)
Cameroon		125 (28.5)		65 (34.8)		60 (23.9)
Vietnam		111 (25.3)		51 (27.3)		60 (23.9)
Underweight (weight for age Z score <-2)	429	276	182	106 (52.2)	247	170 (68.8)
Weight for age Z score	429	-2.5 (-3.6 to -1.8)	182	-2.5 (-3.1 to -1.5)	247	-2.5 (-3.5 to -2.0)
Lansky play performance score	432	80 (80 – 100)	186	95 (80 - 90)	246	80 (70 - 90)
Previous TB treatment		57 (13.0)		12 (6.4)		45 (17.9)
On ART at inclusion		172 (39.3)		80 (42.8)		92 (36.7)
CD4 absolute count (cells per µL)	416	463 (53 – 999)	168	576 (104 – 1072)	246	413 (36 – 924)
CD4 percentage	414	14.0 (3.2 – 24.0)	165	15.1 (4.9 – 24.9)	249	13.0 (3.0 – 23.0)
Immune depression**	414		165		249	
Not significant		128 (30.9)		55 (33.3)		73 (29.3)
Mild and advanced		37 (8.9)		15 (9.1)		22 (8.8)
Severe		82 (19.8)		35 (21.2)		47 (18.9)
Very severe		167 (40.3)		60 (36.4)		107 (43.0)
Hemoglobin (g/dL)	427	10.1 (8.5 – 11.5)	178	10.3 (8.9 – 11.5)	249	10.0 (8.2 – 11.4)

Acid-fast bacilli smear positive	426	27 (6.3)	177	1 (0.6)	249	23 (9.2)
Xpert MTB/RIF positive	425	43 (10.1)	177	0	248	43 (17.3)
Culture confirmed tuberculosis	426	45 (10.6)	177	0	249	45 (18.1)
Non-tuberculous mycobacteria positive culture	426	46 (10.8)	177	19 (10.2)	249	27 (10.8)
Chest radiograph consistent with TB	405	273 (67.4)	169	77 (45.6)	236	196 (83.1)
Tuberculin skin test positive	389	32 (8.2)	161	11 (6.8)	228	21 (9.8)
Initiated anti-tuberculosis treatment		241 (55.0)		11 (5.9)		230 (91.6)
Time to anti-tuberculosis treatment (days)	241	7 (5 – 11)	11	9 (6 – 113)	230	7 (5 – 10)

Data are n(%) or median (IQR). ART=antiretroviral treatment. *If n different from N. †Failure to thrive defined as recent deviation in the growth curve or a weight for age Z score < -2SD. **Immunodeficiency categorized using the WHO immunological classification (2006), adding CD4 below 10% as very severe immunodeficiency.

Table 2. Diagnostic accuracy of tuberculosis tests and predictors after full clinical evaluation

	Sensitivity			Specificity			Positive predictive value		Negative predictive value	
	n/N	%	(IC95%)	n/N	%	(IC95%)	%	(IC95%)	%	(IC95%)
Cough										
In past 24 hours	224/250	89.6	(85.8-93.4)	16/187	8.6	(4.5-12.6)	56.7	(51.8-61.6)	38.1	(23.4-52.8)
Any in past 4 wks	235/250	94.0	(91.1-96.9)	12/187	6.4	(2.9-9.9)	57.3	(52.5-62.1)	44.4	(25.7-63.2)
Lasting >2 wks	212/250	84.8	(80.3-89.3)	35/187	18.7	(13.1-24.3)	58.2	(53.2-63.3)	47.9	(36.5-59.4)
Lasting >3 wks	151/250	60.4	(54.3-66.5)	78/187	41.7	(34.6-48.8)	58.1	(52.1-64.1)	44.1	(36.8-51.4)
Lasting >4 wks	130/250	52.0	(45.8-58.2)	96/187	51.3	(44.2-58.5)	58.8	(52.3-65.3)	44.4	(37.8-51.1)
Unremitting cough	65/250	26.0	(20.6-31.4)	152/187	81.3	(75.7-86.9)	65.0	(55.7-74.3)	45.1	(39.8-50.4)
Fever										
In past 24 hours	148/250	59.2	(53.1-65.3)	112/187	59.9	(52.9-66.9)	66.4	(60.2-72.6)	52.3	(45.6-59.0)
Any in past 4 wks	209/250	83.6	(79.0-88.2)	64/187	34.2	(27.4-41.0)	63.0	(57.8-68.1)	61.0	(51.6-70.3)
Lasting >2 wks	163/250	65.2	(59.3-71.1)	124/187	66.3	(59.5-73.1)	72.1	(66.3-78.0)	58.8	(52.1-65.4)
Lasting >3 wks	99/250	39.6	(33.5-45.7)	151/187	80.7	(75.1-86.4)	73.3	(65.9-80.8)	50.0	(44.4-55.6)
Lasting >4 wks	82/250	32.8	(27.0-38.6)	161/187	86.1	(81.1-91.1)	75.9	(67.9-84.0)	48.9	(43.5-54.3)
Abdominal pain >2 wks	43/213	20.2	(14.8-25.6)	129/150	86.0	(80.4-91.6)	67.2	(55.7-78.7)	43.1	(37.5-48.8)
Loss of appetite in past 4 wks	137/250	54.8	(48.6-61.0)	109/187	58.3	(51.2-65.4)	63.7	(57.3-70.1)	49.1	(42.5-55.7)
Loss of appetite lasting >2 wks	67/250	26.8	(21.3-32.3)	162/187	86.6	(81.8-91.5)	72.8	(63.7-81.9)	47.0	(41.7-52.2)
Chest pain in past 4 wks	48/206	23.3	(17.5-29.1)	112/141	79.4	(72.8-86.1)	62.3	(51.5-73.2)	41.5	(35.6-47.4)
Diarrhea in past 4 wks	76/250	30.4	(24.7-36.1)	134/186	72.0	(65.6-78.5)	59.4	(50.9-67.9)	43.5	(38.0-49.0)
Dyspnea in past 4 wks	76/247	30.8	(25.0-36.5)	150/186	80.6	(75.0-86.3)	67.9	(59.2-76.5)	46.7	(41.3-52.2)
Fatigue in past 4 wks	132/247	53.4	(47.2-59.7)	111/187	59.4	(52.3-66.4)	63.5	(56.9-70.0)	49.1	(42.6-55.6)
Fatigue lasting >2 wks	99/247	40.1	(34.0-46.2)	131/187	70.1	(63.5-76.6)	63.9	(56.3-71.4)	47.0	(41.1-52.8)
Headaches lasting >2 wks	19/205	9.3	(5.3-13.2)	131/136	96.3	(93.2-99.5)	79.2	(62.9-95.4)	41.3	(35.9-46.7)
Hemoptysis in past 4 wks	13/250	5.2	(2.4-8.0)	182/186	97.8	(95.8-99.9)	76.5	(56.3-96.6)	43.4	(38.7-48.2)
Loss of playfulness in past 4 wks	124/248	50.0	(43.8-56.2)	122/187	65.2	(58.4-72.1)	65.6	(58.8-72.4)	49.6	(43.3-55.8)

Sleep disorders in past 4 wks	57/249	22.9	(17.7-28.1)	162/186	87.1	(82.3-91.9)	70.4	(60.4-80.3)	45.8	(40.6-51.0)
Drenching night sweats in past 4 wks	98/249	39.4	(33.3-45.4)	131/187	70.1	(63.5-76.6)	63.6	(56.0-71.2)	46.5	(40.6-52.3)
Vomitting in past 4 wks	38/250	15.2	(10.7-19.7)	145/186	78.0	(72.0-83.9)	48.1	(37.1-59.1)	40.6	(35.5-45.7)
Weight loss in past 4 wks	178/250	71.2	(65.6-76.8)	81/187	43.3	(36.2-50.4)	62.7	(57.1-68.3)	52.9	(45.0-60.9)
Temperature > 37.8°C	78/250	31.2	(25.5-36.9)	146/182	80.2	(74.4-86.0)	68.4	(59.9-77.0)	45.9	(40.4-51.4)
Tachycardia	42/249	16.9	(12.2-21.5)	168/181	92.8	(89.1-96.6)	76.4	(65.1-87.6)	44.8	(39.8-49.8)
Tachypnea	79/242	32.6	(26.7-38.6)	124/172	72.1	(65.4-78.8)	62.2	(53.8-70.6)	43.2	(37.5-48.9)
Chest wall indrawing	36/249	14.5	(10.1-18.8)	173/185	93.5	(90.0-97.1)	75.0	(62.8-87.3)	44.8	(39.9-49.8)
Abnormal lung sounds auscultation	149/249	59.8	(53.8-65.9)	79/187	42.2	(35.2-49.3)	58.0	(51.9-64.0)	44.1	(36.9-51.4)
Reduced breath sounds	50/246	20.3	(15.3-25.4)	145/186	78.0	(72.0-83.9)	54.9	(44.7-65.2)	42.5	(37.3-47.8)
Crackles	119/249	47.8	(41.6-54.0)	101/187	54.0	(46.9-61.2)	58.0	(51.3-64.8)	43.7	(37.3-50.1)
Rhonchus	69/249	27.7	(22.2-33.3)	152/187	81.3	(75.7-86.9)	66.3	(57.3-75.4)	45.8	(40.4-51.1)
Wheezing	18/249	7.2	(4.0-10.4)	173/187	92.5	(88.7-96.3)	56.3	(39.1-73.4)	42.8	(38.0-47.6)
Dullness on percussion	36/249	14.5	(10.1-18.8)	166/186	89.2	(84.8-93.7)	64.3	(51.7-76.8)	43.8	(38.8-48.8)
Any lymph node	92/248	37.1	(31.1-43.1)	132/187	70.6	(64.1-77.1)	62.6	(54.8-70.4)	45.8	(40.1-51.6)
Cervical lymph node	78/247	31.6	(25.8-37.4)	136/184	73.9	(67.6-80.3)	61.9	(53.4-70.4)	44.6	(39.0-50.2)
Hepatomegaly	99/246	40.2	(34.1-46.4)	133/183	72.7	(66.2-79.1)	66.4	(58.9-74.0)	47.5	(41.7-53.3)
Splenomegaly	47/248	19.0	(14.1-23.8)	157/183	85.8	(80.7-90.9)	64.4	(53.4-75.4)	43.9	(38.7-49.0)
Abdominal distension	48/246	19.5	(14.6-24.5)	164/187	87.7	(83.0-92.4)	67.6	(56.7-78.5)	45.3	(40.2-50.4)
Abdominal tenderness	32/248	12.9	(8.7-17.1)	171/187	91.4	(87.4-95.5)	66.7	(53.3-80.0)	44.2	(39.2-49.1)
Quantiferon TB Gold In-Tube positive										
Without indeterminate	39/180	21.7	(15.6-27.7)	129/142	90.8	(86.1-95.6)	75.0	(63.2-86.8)	47.8	(41.8-53.7)
Indeterminate = negative	39/244	16.0	(11.4-20.6)	163/176	92.6	(88.7-96.5)	75.0	(63.2-86.8)	44.3	(39.2-49.4)
Contact with smear+ TB case	24/251	9.6	(5.9-13.2)	183/187	97.9	(95.8-99.9)	85.7	(72.8-98.7)	44.6	(39.8-49.4)
Ultrasonography										
Abdominal lymph nodes	84/237	35.4	(29.4-41.5)	144/168	85.7	(80.4-91.0)	77.8	(69.9-85.6)	48.5	(42.8-54.2)
Chest radiograph										
Ghon focus	10/242	4.1	(1.6-6.6)	175/175	100	(100-100)	100	(100-100)	43.0	(38.2-47.8)
Excavation	10/242	4.1	(1.6-6.6)	174/174	100	(100-100)	100	(100-100)	42.9	(38.0-47.7)

Miliary	14/242	5.8	(2.8-8.7)	170/175	97.1	(94.7-99.6)	73.7	(53.9-93.5)	42.7	(37.9-47.6)
Paratracheal nodes	31/242	12.8	(8.6-17.0)	171/175	97.7	(95.5-99.9)	88.6	(78.0-99.1)	44.8	(39.8-49.8)
Tracheal compression	4/242	1.7	(0.0-3.3)	175/175	100	(100-100)	100	(100-100)	42.4	(37.6-47.1)
Peri-hilar lymph nodes	89/242	36.8	(30.7-42.9)	154/175	88.0	(83.2-92.8)	80.9	(73.6-88.3)	50.2	(44.6-55.8)
Nodular opacities	24/242	9.9	(6.2-13.7)	163/175	93.1	(89.4-96.9)	66.7	(51.3-82.1)	42.8	(37.8-47.8)
Alveolar opacity	94/242	38.8	(32.7-45.0)	134/175	76.6	(70.3-82.8)	69.6	(61.9-77.4)	47.5	(41.7-53.3)
Pleural effusion	13/242	5.4	(2.5-8.2)	167/175	95.4	(92.3-98.5)	61.9	(41.1-82.7)	42.2	(37.3-47.0)
Bronchial compression	23/242	9.5	(5.8-13.2)	175/175	100	(100-100)	100	(100-100)	44.4	(39.5-49.3)
Gibbus	0/240	0.0	(0.0-0.0)	174/174	100	(100-100)			42.0	(37.3-46.8)
Any lymph node	93/242	38.4	(32.3-44.6)	152/175	86.9	(81.9-91.9)	80.2	(72.9-87.4)	50.5	(44.8-56.1)
Any compression	26/242	10.7	(6.8-14.6)	175/175	100	(100-100)	100	(100-100)	44.8	(39.8-49.7)

Table 3. Predictors associated with the diagnosis of tuberculosis in the case control analysis

Predictor	β^*	OR	(95%CI)	P-Value
Fever lasting > 2 weeks	1.0233	2.78	(0.88 – 8.81)	0.082
Hepatomegaly	1.3755	3.96	(1.12 - 14.01)	0.033
Tachycardia	1.9643	7.13	(1.44 – 35.34)	0.016
Abdominal lymph node on ultrasonography	1.6042	4.97	(1.39 - 17.76)	0.014
Splenomegaly on ultrasonography	1.7579	5.80	(0.84 – 40.15)	0.075
Miliary feature on CXR	2.6038	13.52	(1.18 – 154.39)	0.036
QFT result				<0.0001
Positive	3.5726	35.61	(8.08 – 156.92)	
Indeterminate	1.6033	4.97	(1.18 - 20.89)	

*Intercept (constant)=-4.709

Table 4. Predictions models integrating Xpert MTB/RIF results

Predictor	Model 1 with QFT and abdominal ultrasonography				Model 2 with abdominal ultrasonography (no QFT)				Model 3 with QFT (no abdominal ultrasonography)				Model 4 (no QFT or abdominal ultrasonography)			
	β^*	OR	(95%CI)	P-Value	β^\dagger	OR	(95%CI)	P-Value	β^{**}	OR	(95%CI)	P-Value	β^\ddagger	OR	(95%CI)	P-Value
Xpert MTB/RIF				0.0073				0.0061				0.0065				0.0052
Negative		1				1				1				1		
Positive	3.960	52.46	(2.90 – 948.60)		4.127	62.03	(3.25 – >999.99)		3.868	47.833	(2.95 - 775.81)		4.052	57.52	(3.35 – 986.52)	
Fever lasting more than 2 weeks				<0.0001				0.0001				0.0003				0.0003
No		1				1				1				1		
Yes	1.152	3.16	(1.78 – 5.63)		1.137	3.13	(1.76 – 5.53)		1.031	2.81	(1.61 - 4.88)		1.025	2.79	(1.61 – 4.84)	
Unremitting cough				0.0462				0.0592								0.0650
No		1				1				1		0.053		1		
Yes	0.713	2.04	(1.01 – 4.11)		0.671	1.96	(0.97 – 3.93)		0.670	1.95	(0.99 - 3.85)		0.634	1.89	(0.96 – 3.70)	
Hemoptysis in previous 4 weeks				0.0949				0.1174				0.154				0.1812
No		1				1				1				1		
Yes	1.449	4.26	(0.78 – 23.33)		1.358	3.89	(0.71 – 21.26)		1.2080	3.35	(0.64 - 17.62)		1.133	3.10	(0.59 – 16.34)	
Weight loss in previous 4 weeks				0.2440				0.1863								0.0876
No		1				1				1				1		
Yes	0.369	1.45	(0.78 – 2.69)		0.414	1.51	(0.82 – 2.80)		0.482	1.62	(0.89 - 2.95)	0.116	0.519	1.68	(0.93 – 3.05)	
Contact with smear + TB case				0.0170				0.0142								0.0342
No		1				1				1				1		

Yes	1.930	6.89	(1.41 – 33.65)		2.027	7.59	(1.50 – 38.37)		1.624	5.07	(1.08 - 23.85)	0.040	1.708	5.52	(1.14 – 26.78)	
Tachycardia				0.1020				0.0737								0.0781
No		1				1				1				1		
Yes	0.849	2.34	(0.85 – 6.46)		0.925	2.52	(0.92 – 6.95)		0.814	2.26	(0.84 - 6.06)	0.107	0.890	2.43	(0.91 – 6.55)	
Miliary on CXR				0.0915				0.0564								0.0921
No		1				1				1				1		
Yes	1.370	3.93	(0.80 – 19.31)		1.544	4.68	(0.96 – 22.86)		1.156	3.18	(0.68 - 14.76)	0.141	1.317	3.73	(0.81 – 17.30)	
Alveolar opacities on CXR				0.0003				0.0001								0.0009
No		1				1				1				1		
Yes	1.200	3.32	(1.73 – 6.38)		1.280	3.60	(1.88 – 6.88)		0.978	2.66	(1.42 - 4.96)	0.002	1.046	2.85	(1.53 – 5.28)	
Lymph nodes on CXR				<0.0001				<.0001								<.0001
No		1				1				1				1		
Yes	1.771	5.88	(2.98 – 11.58)		1.715	5.56	(2.85 – 10.83)		1.920	6.82	(3.54 - 13.15)	<0.0001	1.863	6.44	(3.37 – 12.30)	
Abdominal lymph nodes on ultrasonography				0.0002				0.0003								
No		1				1										
Yes	1.281	3.60	(1.82 – 7.11)		1.250	3.49	(1.73 – 6.83)									
QFT result				0.1624												
Negative		1								1						
Positive	0.792	2.21	(0.78 – 6.25)						0.714	2.04	(0.77 - 5.45)	0.207				
Indeterminate	0.509	1.66	(0.83 – 3.35)						0.450	1.57	(0.79- 3.11)					

*intercept (constant)= -2.3555; † intercept=-2.1993; **intercept=-1.9602; ‡intercept=-1.8234

Figure 1. ROC curves for the diagnostic prediction models with Xpert

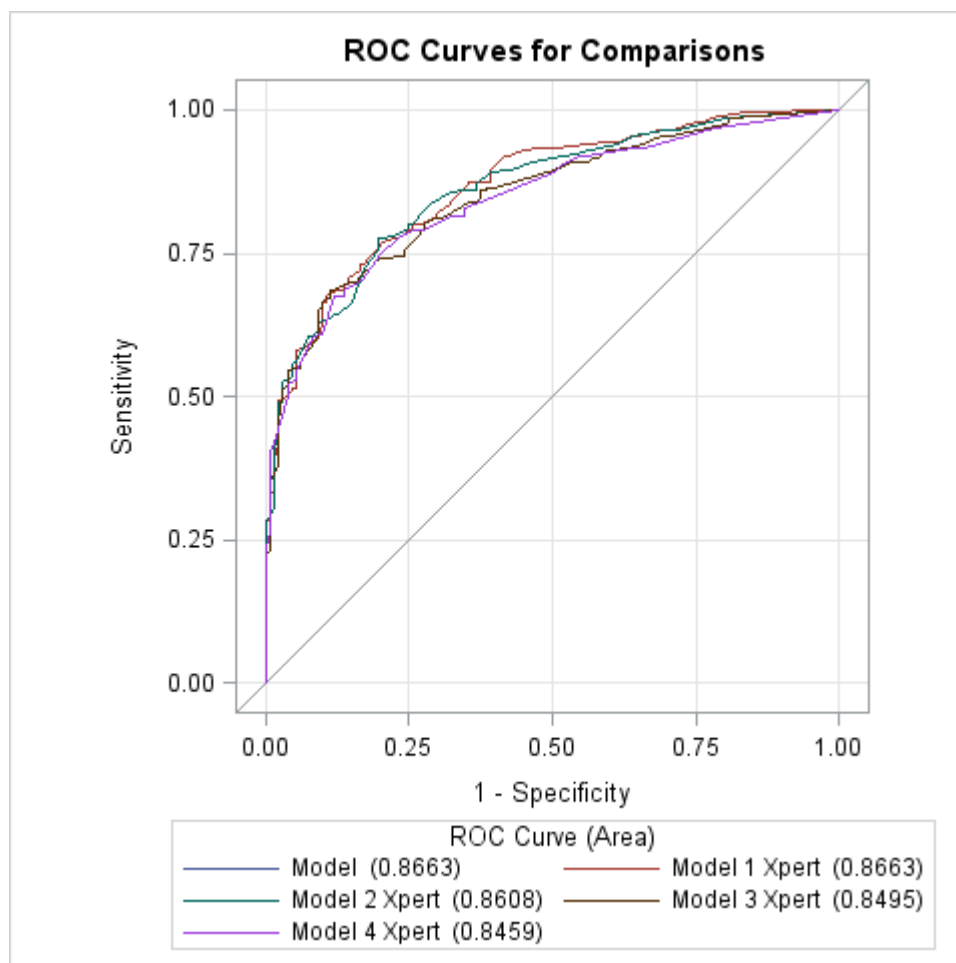
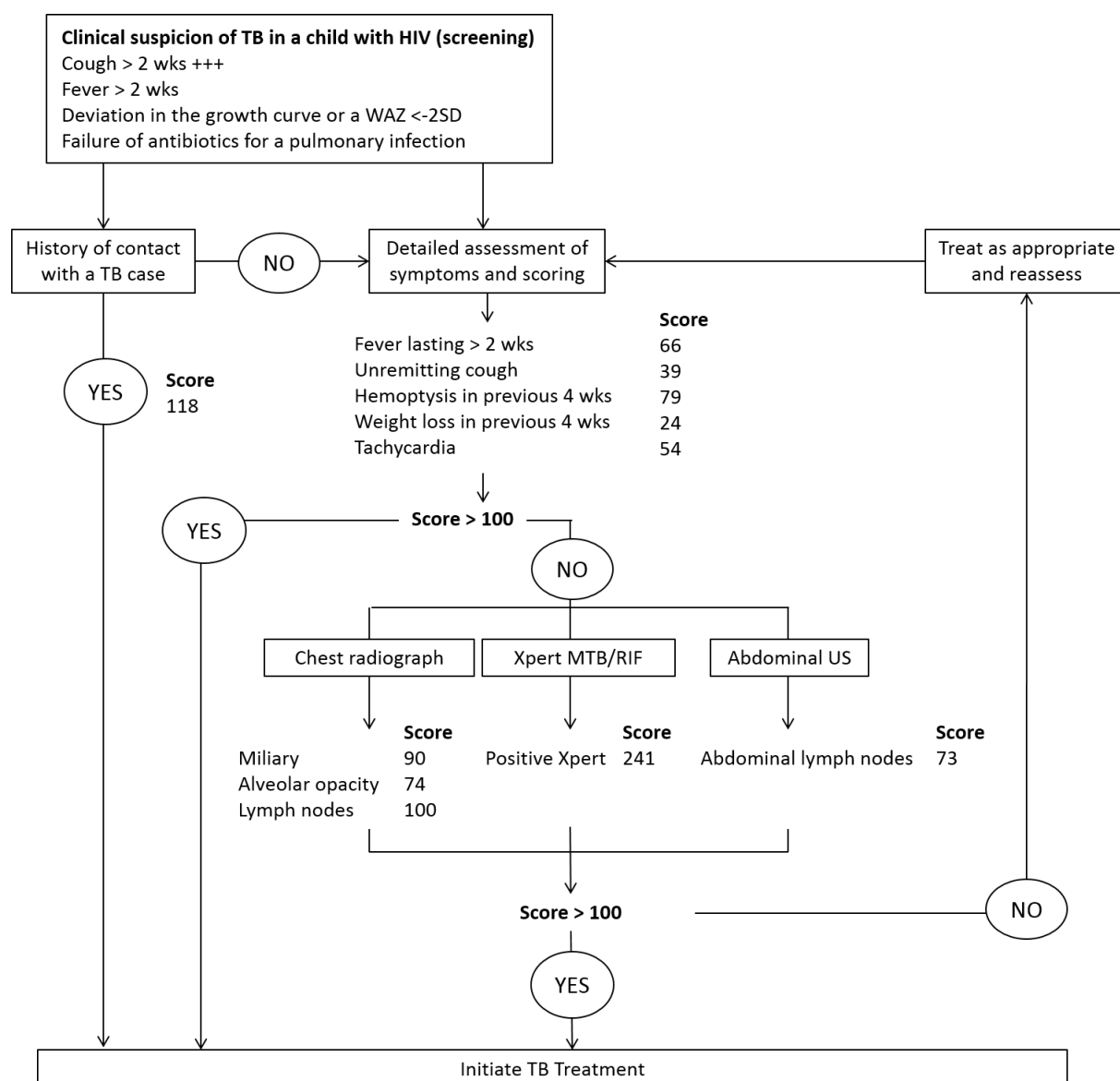


Figure 2. Proposed PAANTHER algorithm including the diagnostic score



7 Use of Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in HIV-Infected Children

7.1 Research question and background

Several composite reference standards have been used or proposed for tuberculosis diagnostic studies in children to overcome the lack of a microbiological gold standard (28, 93, 100, 109, 178, 249). In addition to culture-confirmed cases, standards in individual studies included different approaches to chest radiography, considering it either as a standalone test enabling to diagnose radiographically certain tuberculosis cases, or as a component contributing to classify children as tuberculosis in conjunction with clinical factors or treatment response.

The heterogeneity of reference standard rendered hardly comparable groups defined on different classification systems. A consensus Clinical Case Definitions for Classification of Intrathoracic Tuberculosis Disease specifically designed as a reference standard for research was proposed in 2012 by a group of international experts under the auspices of the US National Institutes of Health (179). It consisted in a retrospective classification system which included treatment response and microbiological confirmation using culture and classified children in 5 groups, considering tuberculosis as confirmed, probable, possible, unlikely, or excluding it as not-tuberculosis. It has been updated in 2015 to include the new molecular diagnostic tool Xpert MTB/RIF as an additional mean of microbiological confirmation, and to take into accounts limitations highlighted in subsequent validation studies (239, 250). The new classification has been simplified and now includes 3 groups, considering tuberculosis as confirmed, unconfirmed, or unlikely (65). None of the two versions of the Clinical Case Definition for Classification of Intrathoracic Tuberculosis Disease have been validated in HIV-infected children.

The ANRS 12229 PAANTHER 01 study incorporated in its study protocol the use of the 2012 version of the Clinical Case Definition, after its publication, and recently used the updated version for the analysis phase. Tuberculosis defined as confirmed or unconfirmed versus unlikely as defined by the 2015 version of the Clinical Case Definition was considered the reference standard to design the diagnostic score developed recently.

We aimed at assessing the impact of using the updated Clinical Case definition on the definitive tuberculosis status of children, to describe how individual characteristics of children contributed to the changes in the tuberculosis status, and to assess factors associated to treatment initiation which is an important contributor to classification by the mediation of treatment response.

7.2 Additional methods

We included in this study on use of Clinical Case Definitions all children with a suspicion of tuberculosis enrolled in the ANRS 12229 PAANTHER 01 study. Study design, inclusion criteria, and procedures have been described in paragraph 2.6 of this thesis.

Definitions

We applied the 2012 and the 2015 version of the Clinical Case Definition for Classification of Intrathoracic Tuberculosis Disease retrospectively to all children enrolled in the cohort (65, 179). This was done applying automatic computerized classification based on pre-defined criteria. In the update Clinical Case Definition, we defined treatment response as clinical improvement under treatment and spontaneous improvement as clinical improvement without tuberculosis treatment. Clinical

improvement was defined as a weight gain $\geq 10\%$ of body weight as compared to baseline at any visit during the first 3 months, and/or an improvement in respiratory symptoms and signs, and/or an improvement in the patient general condition reported by the attending physician. Other definitions are detailed in Appendix 5.

CXR were considered consistent with tuberculosis if reviewers agreed on the presence and location (right/left) of ≥ 1 lesion among the following: alveolar opacity, bronchial compression, excavation, ghon focus, gibbus, miliary, nodular infiltrates, paratracheal nodes, peri-hilar nodes, pleural effusion, and tracheal compression (179).

Statistical analysis

We described characteristics of the groups using frequency and proportion or median and interquartile range, as appropriate. We assessed how missing data contributed to the classification, and modifications in classification that would occur from including QFT in addition to TST in the definition of immunologic evidence of MTB infection.

We assessed inter-classification agreement for the diagnosis of tuberculosis considering confirmed and defined tuberculosis as "tuberculosis" and all other categories as "not tuberculosis" for the 2012 Clinical Case Definition and confirmed and unconfirmed tuberculosis as "tuberculosis" and unlikely tuberculosis as "not tuberculosis" for the 2015 updated version, using kappa statistics.

Additionally, we assessed factors associated with tuberculosis treatment initiation, an important contributor to patient classification, using logistic regression. We included in the multivariate model all variables associated with the outcome at a threshold of 0.20 in univariate analysis. We obtained the final model by a manual backward stepwise process.

We performed analysis using the SAS software version 9.3 (SAS Institute, Cary, NC, USA). A p-value < 0.05 was considered significant.

7.3 Results

Of 438 children enrolled in the study, 55 (12.6%) had tuberculosis confirmed either by mycobacterial culture or by the Xpert MTB/RIF, 196 (44.7%) were classified as unconfirmed tuberculosis, and 187 (42.7%) as unlikely tuberculosis. Their characteristics and factors contributing to their classification are presented in table 4.

In the unlikely tuberculosis group, 10 children had missing microbiological data and could therefore have been misclassified as unlikely. 8 of these died or withdrew from the study within 4 days and also had missing CXR data. When considering all missing CXR or those for which reviewers disagreed on the interpretation as consistent with tuberculosis, 13(3.0%) additional children were reclassified from unlikely to unconfirmed tuberculosis. Considering only those with disagreement as consistent with tuberculosis led to reclassify 3 (0.7%) children only from unlikely to unconfirmed tuberculosis.

When considering positive QFT results as immunological evidence of MTB infection, 33 (7.5%) additional children were considered as having close tuberculosis exposure or immunologic evidence of MTB infection in addition to 60 (13.7%) initially categorized on the basis of TST and history of contact only. However, this had no impact on the classification of children as either unconfirmed or unlikely tuberculosis, which remained similar.

Table 4. Characteristics of children enrolled in the study according to their tuberculosis reference diagnosis using the updated Clinical Case Definition for Classification of Intrathoracic Tuberculosis Disease

	Tuberculosis reference diagnosis (Graham 2015)		
	Confirmed tuberculosis (N=55)	Unconfirmed tuberculosis (N=196)	Unlikely tuberculosis (N=187)
Sex male	29 (52.7)	109 (55.6)	82 (43.9)
Age	7.7 (5.3 - 10.1)	6.8 (3.7 - 9.6)	7.3 (2.2 - 9.9)
Country			
Burkina Faso	12 (21.8)	16 (8.2)	35 (18.7)
Cameroon	21 (38.2)	39 (19.9)	65 (34.8)
Cambodia	4 (7.3)	99 (50.5)	36 (19.3)
Vietnam	18 (32.7)	42 (21.4)	51 (27.3)
Weight for age Z score <-2	32 (58.2)	138 (70.4)	106 (56.7)
Inclusion criteria			
Prolonged cough	45 (81.8)	169 (86.2)	156 (83.4)
Prolonged fever	32 (58.2)	124 (63.3)	74 (39.6)
Failure of antibiotics for a pulmonary infection	33 (60.0)	93 (47.4)	66 (35.3)
Suggestive CXR anomaly	22 (40.0)	85 (43.4)	66 (35.3)
Inclusion criteria failure to thrive	22 (40.0)	83 (42.3)	74 (39.6)
Acid-fast bacilli positive microscopy	23 (41.8)	3 (1.5)	1 (0.5)
Missing smear data	0 (0.0)	2 (1.0)	10 (5.3)
Positive Xpert MTB/RIF	43 (78.2)	0 (0.0)	0 (0.0)
Missing/indeterminate Xpert data	1 (1.8)	2 (1.0)	10 (5.3)
Positive smear or Xpert at baseline*	38 (69.1)	3 (1.5)	1 (0.5)
Positive MTB culture	45 (81.8)	0 (0.0)	0 (0.0)
Missing culture results	0 (0.0)	2 (1.0)	10 (5.3)
Symptoms/signs suggestive of tuberculosis	54 (98.2)	196 (100.0)	181 (96.8)
Cough >2 weeks	44 (80.0)	168 (85.7)	152 (81.3)
Fever >2 weeks	35 (63.6)	128 (65.3)	63 (33.7)
Fatigue >2 weeks	25 (45.5)	74 (37.8)	56 (29.9)
Loss of playfulness >2 weeks	26 (47.3)	68 (34.7)	47 (25.1)
Close tuberculosis exposure or immunologic evidence of MTB infection	17 (30.9)	29 (14.8)	22 (11.8)
New definition 2015 (contact < 1 year)	15 (27.3)	25 (12.8)	20 (10.7)
Close tuberculosis exposure	9 (16.4)	17 (8.7)	13 (7.0)
Contact < 1 year	7 (12.7)	12 (6.1)	11 (5.9)
Immunologic evidence of MTB infection (TST)	8 (14.5)	13 (6.6)	11 (5.9)
TST data missing	6 (10.9)	17 (8.7)	26 (13.9)
Quantiferon TB Gold In-Tube positive	26 (47.3)	13 (6.6)	13 (7.0)
QFT data missing or indeterminate results	16 (5.5)	55 (28.0)	45 (24.1)
CXR consistent with TB	35 (63.6)	161 (82.1)	77 (41.2)
Missing CXR or disagreement on CXR	6 (10.9)	9 (4.6)	18 (9.6)
TB treatment	48 (87.3)	182 (92.9)	11 (5.9)
Positive response to TB Tx (2012)	30 (54.5)	145 (74.0)	110 (58.8)
Improvement under TB Tx (2015)	39 (70.9)	172 (87.8)	4 (2.1)
Spontaneous improvement without TB Tx	4 (7.3)	0 (0.0)	159 (85.0)
Evolution at the end of the study			
Alive, in follow-up	38 (69.1)	166 (84.7)	159 (85.0)
Lost to follow-up	1 (1.8)	2 (1.0)	3 (1.6)
Died	16 (29.1)	25 (12.8)	21 (11.2)
Withdrew from the study	0 (0.0)	3 (1.5)	4 (2.1)

Data are n (%) or median (IQR). * Due to the delayed implementation of Xpert MTB/RIF assays in the study, some tests were performed retrospectively on frozen samples and were not available at baseline. CXR: chest radiograph; MTB: *Mycobacterium tuberculosis*; QFT: Quantiferon TB Gold In Tube; TB: tuberculosis; Tx: treatment.

- Reclassification of children between version 2012 and 2015 of the Clinical Case Definition

Cross classification of the children using both version of the clinical case definition is presented in Table 5. In addition to 45 culture-confirmed tuberculosis, 10 children had positive Xpert results and were thus considered as confirmed tuberculosis using the updated Clinical Case Definition, including 3 classified as probable tuberculosis, 4 as possible tuberculosis, and 3 as unlikely or not-tuberculosis according to the 2012 Clinical Case Definition. Most children with probable tuberculosis according to the initial Clinical Case Definition were considered as tuberculosis by the updated version of the classification. Only 10 (6.6%) were classified as unlikely tuberculosis. Their characteristics are presented in table 3. 9 of them had spontaneous improvement of symptoms without tuberculosis treatment which reclassified them automatically as unlikely tuberculosis. Of 132 children classified as possible tuberculosis by the initial case definition, 58 (43.9) were classified as tuberculosis by the updated case definition. Their characteristics are presented in table 3. All children reclassified had only 2 out of 3 criteria needed to be considered as probable tuberculosis and were thus reclassified as unconfirmed tuberculosis by the fact that only 2 criteria are needed to classify as unconfirmed tuberculosis if there is no spontaneous improvement without tuberculosis treatment.

Using the 2012 version of the Case Definition, 197 (45.0%) children were classified as tuberculosis (confirmed or probable), while 251 (57.3%) were considered as tuberculosis (confirmed or unconfirmed) by the updated version. Inter-classification agreement to distinguish “tuberculosis” from “not-tuberculosis” was 363/438, 82.9 %, with a kappa statistic of 0.6670 (95%CI 0.5999 - 0.7341).

Table 5. Reclassification of children between the 2012 and 2015 versions of the Clinical Case Definition for Classification of Intrathoracic Tuberculosis in Children

2015 updated clinical case definition	2012 clinical case definition			
	Confirmed tuberculosis (N=45)	Probable tuberculosis (N=152)	Possible tuberculosis (N=132)	Unlikely and not-tuberculosis (N=109)
	n (%)	n (%)	n (%)	n (%)
Confirmed tuberculosis	45 (100)	3 (2.0)	4 (3.0)	3 (2.8)
Unconfirmed tuberculosis	0	139 (91.4)	54 (40.9)	3 (2.8)
Unlikely tuberculosis	0	10 (6.6)	74 (56.1)	103 (94.4)

Table 6. Characteristics of children reclassified from “tuberculosis” to “not-tuberculosis” or conversely by change from Graham 2012 to 2015 criteria

	Children reclassified		
	As confirmed (N=10)	From probable to unlikely (N=10)	From possible to unconfirmed (N=54)
Sex male	6 (60.0)	7 (70.0)	26 (48.1)
Age	7.3 (1.6 – 10.3)	2.9 (1.6 – 6.3)	6.5 (3.5 – 9.1)
Weight-for-Age Z Score*	-2.3 (-3.8 to -1.2)	-2.2 (-3.2 to -0.8)	-2.4 (-3.4 to -1.5)
Positive smear result	1 (10.0)	(0.0)	3 (5.6)
Positive Xpert result	10 (100.0)	(0.0)	0 (0.0)
Culture result	0 (0.0)	(0.0)	0 (0.0)
Symptoms/signs suggestive of tuberculosis	10 (100.0)	10 (100.0)	54 (100.0)
Cough >2 weeks	10 (100.0)	6 (60.0)	45 (83.3)
Fever >2 weeks	4 (40.0)	2 (20.0)	36 (66.7)
Fatigue >2 weeks	4 (40.0)	2 (20.0)	25 (46.3)
Loss of playfulness >2 weeks	4 (40.0)	1 (10.0)	17 (31.5)
Close tuberculosis exposure or immunologic evidence of MTB infection	2 (20.0)	10 (100.0)	7 (13.0)
Close tuberculosis exposure	2 (20.0)	4 (40.0)	6 (11.1)
Immunologic evidence of MTB infection	(0.0)	7 (70.0)	1 (1.9)
Chest radiograph consistent with tuberculosis	6 (60.0)	10 (100.0)	22 (40.7)
TB treatment	6 (60.0)	1 (10.0)	40 (74.1)
Positive response to TB Tx (2012)- criteria	5 (50.0)	4 (40.0)	25 (46.3)
Spontaneous improvement without TB Tx	4 (40.0)	9 (90.0)	0 (0.0)
Improvement under TB Tx	3 (30.0)	1 (10.0)	31 (57.4)
Evolution			
Alive	7 (70.0)	10 (100.0)	36 (66.7)
Lost to follow-up	0 (0.0)	0 (0.0)	2 (3.7)
Death	3 (30.0)	0 (0.0)	13 (24.1)
Withdraw.	0 (0.0)	0 (0.0)	3 (5.6)

* Available in 53 children reclassified from possible to unconfirmed. MTB: *Mycobacterium tuberculosis*; TB: tuberculosis; Tx: treatment.

- Factors associated with initiation of tuberculosis treatment

In the multivariate analysis, factors that were independently associated with tuberculosis treatment initiation were the country, with children from Cameroon having a much lower chance to be initiated on treatment than children from all other countries, a previous history of tuberculosis disease or lack of information about this history, a CXR consistent with tuberculosis or a lack of agreement from reviewers on the CXR, positive Xpert and/or smear microscopy results, a prolonged fever for more than two weeks, and a Lansky play performance score below 90, which characterizes children with reduced activity (251), which were all associated with a higher chance of being initiated on tuberculosis treatment (Table 7).

Table 7. Factors associated with treatment initiation in children with a suspicion of tuberculosis

	Univariate Analysis			Multivariate Analysis		
	OR	(95%CI)	P-Value	OR	(95%CI)	P-Value
Country			<0.0001			0.0004
Cameroon	1			1		
Burkina Faso	1.366	(0.733 - 2.547)		3.024	(1.307 - 6.993)	
Cambodia	4.976	(2.629 - 9.418)		3.847	(1.976 - 7.488)	
Vietnam	2.046	(1.085 - 3.858)		3.004	(1.505 - 5.996)	
Sex			0.0563			
Male	1					
Female	0.692	(0.474 - 1.010)				
Age (years)			0.2731			
<2	1					
[2 – 5[1.337	(0.689 - 2.596)				
[5 – 10[1.641	(0.968 - 2.783)				
≥10	1.200	(0.664 - 2.172)				
Weight-for-age Z score			0.0222			
≥ -2	1					
[-3 to -2]	1.524	(0.965 - 2.406)				
<-3	1.922	(1.193 - 3.097)				
History of TB disease			<0.0001			0.0126
No	1			1		
Yes	4.182	(2.099 - 8.331)		3.513	(1.475 - 8.367)	
Unknown	3.500	(1.130 - 10.836)		2.090	(0.580 - 7.529)	
On ART at inclusion			0.1334			
No	1					
Yes	0.744	(0.506 - 1.094)				
CD4			0.1686			
≥10%	1					
<10%	1.325	(0.888 - 1.980)				
CXR consistent with TB			<0.0001			<.0001
No	1			1		
Yes	4.351	(2.791 - 6.781)		4.655	(2.673 - 8.105)	
Missing (discordant)	3.548	(1.304 - 9.651)		3.362	(1.010 - 11.185)	
Smear and/or Xpert positive			<0.0001			0.0001
No	1			1		
Yes	19.403	(4.626 - 81.382)		58.229	(7.451 - 455.046)	
Prolonged fever> 2 weeks			<0.0001			0.0005
No	1			1		
Yes	3.173	(2.142 - 4.699)		2.435	(1.474 - 4.021)	
Lansky score						0.0004
≤80	2.965	(2.006 - 4.381)		2.583	(1.528 - 4.365)	
>80	1			1		

ART: antiretroviral treatment; CXR: Chest radiograph; OR: odds ratio; TB: tuberculosis

7.4 Discussion

We used the initial and the updated versions of the Clinical Case Definitions for Classification of Intrathoracic Tuberculosis to classify HIV-infected children enrolled in the ANRS 12229 PAANTHER 01 study with a suspicion of tuberculosis.

Limitations of the 2012 version of the Clinical Case Definition have been highlighted before. It included the fact that chronic symptoms are not always found in children with culture-confirmed

tuberculosis and that the classification should allow for bacteriological confirmation without specific symptoms (239) The second limitation identified was that the Clinical Case Definition was not adapted for household contact tracing (HHCT) research as 69 out 111 children assessed through HCCT in a South African study could not be classified using the 2012 version of the classification (250). This was not surprising as the initial definition was intended to be used in children presenting with symptoms, who may be different from those identified at very early stage of the disease through HHCT. This however highlights the potential weaknesses of such a reference standard and the fact that it should be validated, especially in HIV-infected children.

The inter-classification agreement between version 2012 and version 2015 of the Clinical Case Definition was good. Per protocol, we had decided to design an algorithm that would predict tuberculosis as defined by confirmed and probable tuberculosis. Changing to the updated version of the Clinical Case Definition led to a 28% increase in the number of children considered as tuberculosis. Changes were due to the fact that the new classification requires 2 criteria only for probable tuberculosis, in the absence of spontaneous resolution of symptoms. Clinically, The new disposition taking into account spontaneous resolution of symptoms in the absence of tuberculosis treatment as a dominant criteria classifying in the unlikely group seems clinically justified.

Of 10 children reclassified as confirmed tuberculosis, 7 were considered as possible tuberculosis and unlikely or not tuberculosis according to the 2012 Clinical Case Definition. This raises the question of an imperfect specificity of Xpert rather than of a sensitivity higher compared to culture.

10 children were reclassified from probable tuberculosis to unlikely tuberculosis. All were alive after 6 months of follow-up. All had a CXR consistent with tuberculosis, a close tuberculosis exposure or an immunologic evidence of MTB infection, and signs and symptoms suggestive of tuberculosis. Despite the fact that clinicians did not initiate tuberculosis treatment during the duration of follow-up, this should lead to discuss again classification criteria for unlikely tuberculosis.

The classification relies heavily on the clinician's decision to treat tuberculosis. Very few children without tuberculosis treatment were classified as unconfirmed tuberculosis, and conversely, very few children with tuberculosis treatment were considered as unlikely tuberculosis. The risk is therefore that sites with a tendency to overtreat lead to increase false positive unconfirmed tuberculosis by treating children without tuberculosis, which may have otherwise improved without treatment. It was obvious from early discussion among the PAANTHER working group that practices across countries varied quite widely and that access to tuberculosis drugs for children lacking bacteriological proof was not easy in all sites. Factors associated to tuberculosis treatment initiation are therefore important to understand why clinicians initiated children under treatment and check if there is any sign of overtreatment. We did not detect obvious overtreatment in one site, rather that children in Cameroon had a lower risk to access treatment than children from other countries when adjusting on the previous tuberculosis history of the child, the consistency of his/her CXR with tuberculosis, smear and/or Xpert positive results availability at baseline, and prolonged fever for more than 2 weeks and low Lansky play-performance score which provide additional information about clinical status of the child. Children in Cameroon were quite young and severe and had a high risk of mortality, associated probably to a very deteriorated clinical status and additional unmeasured factors which may have impacted access to tuberculosis treatment and the clinician's decision to start treatment. In any case, none of the factors associated to treatment are a surprise from a clinical point of view and we did not detect an obvious issue of overtreatment in one the sites compared to the other. We cannot exclude however that clinicians from all sites may have been more aware about tuberculosis in the research environment, and therefore encouraged to initiate tuberculosis treatment.

Implementing these classifications has some technical challenges and potential drawbacks. A challenge in using these definitions is that, despite giving precise indication on criteria, these remain always subject to some level of interpretation. A study published recently used the 2015 version of the Clinical Case Definition with criteria different than ours to define clinical improvement (72). They also relied on a single CXR reader which may significantly impact accuracy of this reference reading. As shown in our work on CXR (see § 5), it has poor inter-reader reproducibility and diagnostic accuracy for tuberculosis in HIV-infected children. This has an impact on the classification of children and will tend to over-classify children as confirmed tuberculosis due to the globally poor specificity of CXR lesions. However this may be balanced by the clinician's decision to avoid treating the child for tuberculosis. Use of Xpert as a confirmatory test also raises issues of potential false positive as is seen with molecular techniques in other fields of bacteriology. Specificity of Xpert Ultra is expected to be lower than that of Xpert MTB/RIF in children (252). While it may well be an improvement for tuberculosis diagnosis in children, showing a real reduction in the limit of detection (LOD) as compared to the suboptimal LOD of culture, one cannot completely exclude the hypothesis of real false-positive results as seen in other molecular methods. The classification is quite stable however to changes in use of some criteria such as integrating QFT results or not to define immunologic evidence of MTB infection.

Our study had limitations. First, we did not perform a centralized expert panel review of medical charts of children who died, as it was recommended in the updated version of the Clinical Case Definition, as medical charts contained scarce information in addition to data collected in the study Case Report Form. Attempting to do so on the basis of collected data led to consider most children who died as unlikely because CXR findings were rarely specific (see § 5) and that there was no clinical improvement under treatment to additionally confirm tuberculosis. We felt that this was introducing a differential verification and potential bias in children who died only, and that, in this case, all children's medical charts should be reviewed. Our work on mortality has proven that even treated early and properly, tuberculosis can be lethal in HIV-infected children. Second, a small number of children had missing data which may have biased classification toward more unlikely tuberculosis. Due to the structure of missing data and the number of missing variables, we were unable to include these children in the prediction score dataset and they were not considered in mortality analyses either, which may have biased partially parameter estimates. Sensitivity analysis on the prediction score obtained however show that performances of the score are maintained whether all missing predictors are considered as positive or negative, which is reassuring. At last, by design, we had limited data available concerning differential diagnosis which may have been helpful to balance the clinician's decision to treat for tuberculosis. This however requires reviewing all individual patients' chart, which is challenging with a large number of children enrolled in the study and with multicentric international settings where centralized endpoint review is complex.

We used the updated version the Clinical Case Definitions for Classification of Intrathoracic Tuberculosis as diagnostic reference standard for HIV-infected children enrolled in the PAANTHER 01 study. Compared to the initial version of the Clinical Case Definition, it considered more children as tuberculosis as approximately half of children initially classified as possible tuberculosis were classified as unconfirmed while the other half was classified as unlikely. The addition of spontaneous symptom resolution as a strong criterion in the classification reinforces the confidence in the "unlikely" category as being children without tuberculosis. This however accentuates the importance of the clinician's treatment decision in the study and probably the need to standardize as much as possible such decision across sites in multicentric studies. Our analysis of factors associated with tuberculosis treatment decision seems to identify under-treatment in one site rather than over-treatment

that would lead to increase false positive unconfirmed tuberculosis. The lack of specificity of this reference standard cannot however be excluded, and overtreatment may well remain a persistent issue across sites and clinical practices, as suggested by a latent class Bayesian analysis of pediatric tuberculosis data which revised tuberculosis prevalence downward compared to clinicians (253). Missing data and imprecise definitions make the use of these standards still challenging.

8 General discussion

The ANRS 12229 PAANTHER 01 was a large and unique prospective cohort of HIV-infected children with a suspicion of tuberculosis. A large number of studies, especially from Southern African countries, addressing different questions on the diagnosis of tuberculosis in children include HIV-infected children but, to our knowledge, this is the first diagnostic study exclusively focusing on HIV-infected children.

Study findings and public health implications

- Mortality in HIV-infected children with a suspicion of tuberculosis

HIV-infected children present specific challenges as compared to uninfected children. In HIV-infected children one of the major challenges is the deadly synergy between the two infections due to HIV and MTB. This translates in a very high risk of mortality despite proper access to treatment, as shown in our analysis of mortality and its determinant in ART-naïve children. In this study ART initiation was significantly delayed in children initiated on tuberculosis treatment. Together with our findings on Xpert positivity as a strong risk factor of mortality and the impact of tuberculosis treatment initiation on mortality in children with both confirmed and unconfirmed tuberculosis, these show the importance of tuberculosis diagnosis in clinical management of HIV-infected children with a suspicion of tuberculosis.

Timing of ART plays a major role in reducing mortality in HIV-infected children with presumptive tuberculosis, whether they are initiated or not on tuberculosis treatment. WHO's recommendation to initiate ART between 2 to 8 weeks after anti-tuberculosis treatment start and earlier when CD4 are below 50 cells/ μ L was based on data from adult trials (254-256). There is no clinical trial data available addressing specifically the question of timing of ART in children treated for tuberculosis. Cohort data available should be interpreted cautiously. A study from Malawi showed a 9-fold increase in mortality in children not initiated on ART, as compared with those that were started on ART during the first two months of follow-up (240) while another study assessing impact of timing of ART on mortality in South-African children treated for tuberculosis concluded that ART initiation delayed by 2 to 4 weeks after anti-tuberculosis treatment start did not increase mortality (257). However both studies had limitations. The study from Malawi used logistic regression to identify factors associated with mortality and therefore did not take into account the effect of time and the fact that potentially children may have died before introduction of ART was feasible. The South African study had a much lower initial mortality but a higher rate of loss to follow-up which may have masked early deaths. The use of a time-dependent variable for analysis of the effect of ART in our study enabled to show the importance of very early ART, although we were not able to completely exclude that there may be other unmeasured co-factors which contributed to the difference in mortality. We initially attempted to use propensity scores but these were unable to take into account the time off ART, which seems to be the biggest mortality risk factors. Our findings suggests that clear recommendations should be given to introduce ART early in children with severe criteria, probably within 2 weeks of ART initiation, as recommended in adults with CD4 below 50 cells/ μ L, or even earlier. A third of children from the South African cohort initiated ART concomitantly with anti-tuberculosis treatment (257). At this stage however, such a recommendation cannot be done formally, especially in children with positive Xpert or those with disseminated tuberculosis. Indeed, despite reports of much lower rates of paradoxical tuberculosis-associated IRIS in children than in adults, significant IRIS-related morbidity could be an issue in children, especially if those have sub-clinical abdominal or neurological tuberculosis localization (28, 258-260).

Our results suggest optimizing the timing of both ART and tuberculosis treatment initiation in tuberculosis/HIV co-infected children. Two possible ways to shorten the time to tuberculosis treatment decision and therefore time to ART initiation are: 1) to optimize and shorten microbiological confirmation by the use of more adapted specimen collection methods, 2) to develop an algorithm for empirical treatment decision in children which are not bacteriologically confirmed. Both questions have been addressed in the framework of the ANRS 12229 PAANTHER 01 study and this thesis.

- Performance of Xpert on NPA and stool samples

Despite the limited yield of bacteriological confirmation, attempting to confirm the diagnosis by microbiological tests remains crucial. This is particularly true as the tests that are available now enable concomitant tuberculosis diagnosis and identification of the *rpo B* mutations conferring resistance to rifampin (61). With the growing incidence of tuberculosis drug resistance at global level (1) and major challenges in detecting and initiating patients on adapted second line treatment, early detection of rifampin resistance most often associated to isoniazid resistance and therefore multidrug resistance is of major importance. Xpert positive children also have a higher risk of mortality, as shown in our study, and this should trigger prompt tuberculosis treatment.

Pediatric microbiological sample collection methods currently recommended by the WHO are scarcely implemented. Gastric aspirates are quite invasive and require hospitalization and proper containment which is requiring in terms of human resources as 2 to 3 nurse or assistants are needed to properly contain the child and perform sample collection. Gastric aspirates are usually repeated 3 days in a row, require overnight fasting and optimally to be performed early morning in supine position which usually require hospitalization. As a result most sites rely on expectorated sputum samples which are suboptimal in children above 5 and completely inefficient in those below the age of 5 (96). Adapted and child friendly sample collection methods are therefore needed. Our study on alternative specimen collection methods and their use with Xpert MTB/RIF brings evidence on the interest of these methods and their possible use in a one-day child-friendly sample collection strategy. Our study shows that despite its very good tolerability and acceptability by children which were able to swallow the capsule, the string test was disappointing both in terms of feasibility and performance. Nasopharyngeal aspirates however, as stand alone or in combination with stool samples, had both very high feasibility, above 98%, and a good diagnostic performance. Our findings about the good diagnostic performance of Xpert MTB/RIF performed on a combination of stool and nasopharyngeal aspirate, which led to at least one sample collected in 100% and each sample collected in 98% of children in the 272 children enrolled in our Xpert study, have been confirmed since by Rinn Song et al (ref Song conference) in the Toto study which included children below the age of 5 years in Kisumu Kenya.

Several studies have since reported findings on the use of Xpert on stool samples for the diagnosis of tuberculosis (72, 261, 262). They confirmed the interest of stool samples for microbiological diagnosis, despite a sub-optimal sensitivity of stool samples compared to respiratory samples. Most studies however relied on simple processing methods including dilution with PBS and simple centrifugation methods. None used a flotation method with Sheather's solution, as we used in the PAANTHER study. Despite controversial data regarding the interest of flotation methods, the high sensitivity and very low rate of indeterminate tests in our study support the use of this method (263). Banada *et al* used a DNA extraction method using Qiagen reagents and showed a very high yield in children with culture negative probable tuberculosis (94). Whether these results will be maintained in the kit currently developed by David Alland and FIND warrants further evaluation.

- Diagnostic score and algorithm

To enable a timely diagnosis of tuberculosis, the diagnostic process needs to be decentralized. Sophisticated diagnostic approaches based on physiopathology are adapted for specialized services at tertiary level but not adapted to lower level of care with limited capacity and clinical skills (17). Scoring systems extensively used and previously recommended by the WHO (139) have been abandoned in recent guidance on childhood tuberculosis diagnosis (7, 8, 158), due to heterogeneous performances of diagnostic approaches, lack of validation, and poor performance in specific populations such as HIV-infected children, and those with severe acute malnutrition (100, 136). However, to enable integration of tuberculosis diagnosis at lower level of care, the approaches need to be standardized and simplified, eventually to a level similar to Integrated Management of Childhood Illnesses (IMCI). The diagnostic algorithm developed in our work on the score enables a simplified and stepwise approach to the diagnosis of tuberculosis in HIV-infected children.

Interestingly, most variables included in our score have been used in diagnostic approaches developed using expert opinion. In addition to the Xpert MTB/RIF test included in the approach, it includes predictors that can be assessed relatively simply through an interview with a parent/guardian of the child as symptoms and their duration, a simple sign that can be assessed through physical examination, and chest radiograph findings which are most often largely available at HIV outpatient follow-up sites or hospital. Availability of QFT and abdominal ultrasound are however more problematic. WHO has issued strong recommendations against the use of QFT due to its lack of capacity to distinguish between LTBI and tuberculosis disease (264) which has limited its dissemination in low income countries. Ultrasound machine may not be available in remote settings. Training of primary care clinician for the detection of abdominal lymph nodes is possible as demonstrated previously (265). The developing interest for ultrasonography as an alternative to CXR for the diagnosis of pneumonia may contribute to a broader diffusion of this exam (266).

Optimally, tuberculosis diagnosis in children should be possible within one to two days at most. NPA can be performed on the spot without overnight fasting. Stools however cannot be produced on demand, and it may take more time to recover a sample, probably in the next morning. Due to its design, our algorithm should enable prompt treatment decision in children with typical clinical features. Access to ultrasonographic exam and CXR could be done within the same day, providing they are readily available in the healthcare facility.

With its high sensitivity and algorithmic approach the score developed in the PAANTHER study should enable to initiate treatment rapidly in most children at risk of tuberculosis-related mortality in those presenting with presumptive tuberculosis, thus contributing to significant public health benefits. We chose to favor sensitivity rather than specificity in a consequentialist approach of the diagnosis, in order to reduce tuberculosis related mortality in these children. Despite concerns raised regarding this low specificity and potential overtreatment, the net benefit of the score in the subpopulation at high risk of mortality outweighs probably the harms associated with false positive diagnosis. Indeed tuberculosis treatment is well tolerated in most children (267). Efficacy of efavirenz with a NRTI backbone taken concomitantly with rifampin is also good (268), despite variable effect of rifampin as cytochrome P450 inducer, accelerating the metabolism and elimination of NNRTIs and protease inhibitors (269). We used internal validation to assess the practical diagnostic value of the proposed score in real life. However, this process does not enable refining of the score. To enable assessing its clinical usefulness and adapting both the scoring system and the overall approach, an external validation is needed.

In addition to the diagnostic performance of the algorithm itself, there are pending questions, including how to integrate the algorithm with the WHO recommendation to assess for tuberculosis any child with fever, current cough, weight loss, and history of contact (40). These signs are very straightforward to collect in routine, although operationalizing systematic sign collection and adding up this screening tool on the task list of overburdened healthcare providers is a challenge. They were recommended by the WHO on the basis of a still unpublished study (Rinn Song, IAS conference 2012). The screening strategy in adults has been recommended on the basis of a meta-analysis including among 12 observational studies including one major study which selected the best 3 predictors among 80 million of combinations of 100 potential predictors, without internal validation (270). This study implemented in South-East Asia showed that the presence of cough of any duration, fever of any duration, or night sweats lasting 3 or more weeks in the preceding 4 weeks was 93% sensitive and 36% specific for tuberculosis.

The second major question pending is to know in what measure IPT can be recommended, and when it can be recommended, in symptomatic children testing negative or tuberculosis with our algorithm. The WHO currently recommends initiating on IPT all HIV-infected children that enter care and who do not present fever, cough, weight loss, or history of contact with a tuberculosis case and to consider it in symptomatic children if tuberculosis has been ruled out (40).

- Limitations of chest radiography for the diagnosis of tuberculosis in HIV-infected children

Chest radiography plays a key role both as a diagnostic test and also as a contributor to the composite reference standard for tuberculosis diagnostic studies. In HIV-infected children, frequent comorbidities and opportunistic infections contribute to reduce the specificity of CXR lesions consistent with tuberculosis. As shown inter-reader agreement is poor for lesions which are consistent with tuberculosis, as suggested by Graham *et al* (179). In our study, poor inter-reader agreement contributes to reduce sensitivity and increase specificity. This latter however remains low, especially in children with severe immunodeficiency. Children who remained in follow-up after 6 months without tuberculosis treatment may still present CXR features which are consistent with tuberculosis, whether these are quite specific for tuberculosis, such as miliary, which can also be indicative of lymphocytic interstitial pneumonia, or non-specific such as alveolar opacities, which are frequent in children with tuberculosis and not tuberculosis. Unfortunately, due to the lack of local endpoint validation committees and absence of standardized assessment of other causes of CXR anomalies and lung diseases, information on alternative diagnosis was not available.

Despite these challenges, CXR is able to identify up to 90% of children with culture confirmed tuberculosis. However, in practice, the value of CXR is generally challenged by the poor quality and/or the low interpretation skills of healthcare workers. Training is therefore instrumental to teach clinician to recognize lesions. Training may prove challenging, especially for the detection of perihilar or paratracheal lymph nodes which contributes to the diagnostic score (125, 246). A computer-aided CXR reading enabling proper identification of perihilar adenopathy would be a major advance. Unfortunately, computer-aided CXR reading has not yet been validated for use in children, notably due to the technical difficulties in obtaining a CXR of good quality especially on rotation and inspiration in young children, and slightly less on penetration in case of digitalized CXR (271).

- Use of internationally recommended reference standards for diagnostic studies

At last, we presented and discussed data on the use of the recommended tuberculosis reference standard for diagnostic studies in HIV-infected children, describing changes in classification occurring with the recent update and highlighting limitations of these reference standards. Inter-classification

agreement was good. Classification relies heavily on clinician's decision to treat and therefore may lack specificity, depending of the site's clinical practices. By design, the study collected little information on differential diagnoses in children enrolled in the cohort. Assessment for differential diagnoses was not standardized and was highly dependent upon local capacities and clinical practices. This is one of the major issues with using a tuberculosis only classification in these children, especially as these are HIV-infected and therefore susceptible to have many other opportunistic infections or HIV-associated illnesses.

Methodological considerations

In addition to challenges in interpreting CXR findings that contribute to the diagnosis as well as the reference standard, these studies faced a number of methodological challenges which are frequent in diagnostic studies, particularly tuberculosis diagnostic studies in children, that need to be understood when conducting, analyzing, and interpreting such studies.

- Lack of gold standard and use of a composite reference standard

A major methodological challenge, which is inherent to the nature of childhood tuberculosis, is the lack of a gold standard. Mycobacterial culture is the accepted standard for tuberculosis diagnosis in adults (180). An ideal gold standard for a diagnostic accuracy study should (1) provides error-free classification of all subjects; (2) be used uniformly to verify all index test results; (3) be performed within a short time of the index test to avoid changes in target condition status (272). Due to its much lower sensitivity in children, mycobacterial culture cannot be used as full reference standard. There are several options to assess diagnostic accuracy in the absence of a perfect reference standard (272). This includes the construction of a new reference standard, either by using a second reference standard in those in which the preferred one is not available (differential verification); alternatively, results from a series of tests can be combined to construct the reference standard, based on either (1) a predefined deterministic rule (composite reference standard); (2) consensus among experts (panel diagnosis); (3) or a statistical model based on actual data (latent class analysis). In childhood tuberculosis, the composite reference standard recommended and recently updated is now being used more extensively at international level (65, 72, 179).

Several issues may arise from the use of a composite reference standard. Incorporation bias arises from the use of the index test to determine the reference standard, or conversely, the use of the reference standard to determine the results of the diagnostic test, e.g. in the case of our work on the diagnostic score, CXR features contributed to classify the child for the reference tuberculosis diagnosis and were also used in the index tests. Generally incorporation bias leads to overestimate the diagnostic accuracy of the index test (273). Furthermore, in our study on mortality, since treatment response significantly contributes to classification of children in the different diagnostic categories we were unable to properly assess the impact of tuberculosis treatment in a multivariate analysis.

The use of a composite reference standard may also lead to differential verification or partial verification. Differential verification arises when the reference standard is not assessed in the same way in all study participants. It can lead to biased estimates of diagnostic accuracy. Indeterminate and missing tests such as missing bacteriological tests due to feasibility issues may therefore contribute to differential verification. Differential or partial verification may also lead to overestimate the diagnostic accuracy of the index test (273).

To avoid issues related to the use of composite reference standard, an approach based on latent class analysis can be used (180, 274). Latent class models are approaches considering the target disease

status as an unmeasured (“latent”) categorical variable. Previous use of such methods in children with a suspicion of tuberculosis showed that overdiagnosis is an issue and that prevalence of tuberculosis was lower than assessed clinically (253). However, these methods have many limitations, including the fact that the model assumptions, which cannot be validated, influence heavily the estimation of sensitivity and specificity. In an attempt to better estimate prevalence of tuberculosis in the PAANTHER cohort and to assess the diagnostic accuracy of several tests, independently from the Clinical Case Definition, we performed a preliminary study on the diagnostic accuracy of microbiological methods, QFT, and chest radiography using latent class analysis, which is not presented in the framework of this thesis. Despite good statistical results of the models used with satisfying goodness of fit, the results were clinically disappointing (275). The prevalence of tuberculosis was estimated to be lower than the proportion of bacteriologically confirmed cases, either by Xpert or culture. One of the question raised by this preliminary analysis is whether the specificities of tuberculosis presentation in HIV-infected children, such as the high frequency of normal CXR in children with microbiologically confirmed tuberculosis, the higher rate of CXR features in unconfirmed tuberculosis cases, and the high rates of indeterminate tests in children with lower CD4 counts and higher proportion of positive Xpert, may have contributed to this surprising findings of the latent class model. A new analysis is planned with additional data from a mixed population of HIV-infected and HIV-uninfected children from Mbarara, Uganda, to assess the impact of HIV-infection on the performance of the model.

- Missing data, indeterminate results, and the intention to diagnose principle

One if the questions arising from missing and indeterminate index tests results is how these should be considered when evaluating the diagnostic accuracy of the test. It is common practice in most diagnostic studies to exclude these results when evaluating accuracy (178, 276, 277). This however does not reflect the performance of the test in real life as clinicians will be confronted to this kind of results. Repeating the test may prove unfeasible or may well lead to the same results as the condition leading to indeterminate test may still be prevalent, such as immunodeficiency or young age leading to indeterminate IGRAs. The intention-to-diagnose (ITD) approach and the use of a 2 by 3 table rather than a usual 2 by 2 table have been proposed to better reflect the reality of indeterminate or missing results and their impact on diagnosis accuracy (276). Similarly to the intention to treat principle in clinical trials, where the endpoint is evaluated regardless of whether the patient is still under treatment or not at the time of the evaluation, the ITD consists in incorporating the information of all intended tests in the evaluation of diagnostic accuracy, regardless of whether the tests actually had an interpretable result or not. In a proposed worst case scenario, this leads to consider all indeterminate or missing tests as negative when assessing the sensitivity and as positive when assessing the specificity. In our work on performance of Xpert on alternative samples, we did not apply the worst case scenario and instead chose to consider all missing tests as negative. This can be justified by the fact that all patients had at least one negative sample. The second and third samples usually have little incremental value and with the prevalence of microbiologically proven tuberculosis in our cohort, missing data would potentially have much more impact on sensitivity than on specificity. In the case of a single non-repeated tests such as the QFT test however, a worst case approach would be more justified and should be recommended. Incorporating missing test results in the assessment of diagnostic accuracy of Xpert on alternative specimens showed a major difference in sensitivity of Xpert on string tests between the per protocol and the ITD approach with sensitivity falling down from 62.5% to 36.0%, due to major feasibility issues. The sensitivities of NPA and stool, however, were similar in both approaches, due to the good feasibility of both tests. Sensitivity of NPA alone even seemed slightly higher in ITD, probably due to the fact that children which had missing tests had more severe disease.

The ITD approach to describe tests results should therefore be recommended for diagnostic studies in the field of childhood tuberculosis to account for feasibility issues in proposed tests.

Perspectives

Multiple research questions around diagnosis of tuberculosis in HIV-infected children are still unanswered. The large amount of data collected in the ANRS 12229 study will enable to address additional questions. Ongoing analyses and publications by the PAANTHER working group will address the following topics: 1) diagnosis and clinical management challenges of non-tuberculous mycobacterial infections in HIV-infected children, especially in South East-Asia, 2) tolerability and safety of alternative specimen collection methods, 3) detailed assessment of the diagnostic value of QFT, and 4) latent class analysis of tuberculosis prevalence and diagnostic accuracy of microbiological tests in the PAANTHER cohort and a cohort of HIV uninfected children from Mbarara, Uganda, in collaboration with Epicentre.

A major perspective for the follow-up of the work undertaken in the framework of the ANRS 12229 PAANTHER 01 study and of this thesis is the TB Speed project which will enable to conduct an external validation study for the score/algorithm, and to validate the use of the combination of NPA and stool samples for detection of tuberculosis with the Xpert Ultra, the new generation of Xpert cartridges for tuberculosis diagnosis, in other pediatric populations and in decentralized settings.

The TB-Speed project is a research project co-funded by Unitaïd and the French 5% initiative, led by the Infectious Diseases in Low Income Countries (IDLIC) team of the Bordeaux Population Health research center on behalf of the University of Bordeaux. Its global goal is to contribute to the reduction in childhood mortality from tuberculosis by delivering an available, feasible, cost-effective, and decentralized childhood tuberculosis diagnostic approach to enhance case-finding and access to treatment.

It is a four-year project implemented in seven countries (Cambodia, Cameroon, Côte d'Ivoire, Mozambique, Sierra Leone, Uganda, and Zambia) based on three major patient-centered outputs or work packages and three technical support outputs building evidence for future scaling up. Ultimately, the project will generate evidence on the most appropriate decentralization strategy for tuberculosis diagnosis and the improvement of tuberculosis diagnosis in high-risk children (HIV-infected, malnourished children and children with severe pneumonia)

The innovative nature of the project resides in the validation of a decentralized diagnostic approach using battery-operated GeneXpert OMNI and Xpert ultra cartridge to increase access to tuberculosis diagnostics. It also proposes a simplified sample collection methods (stools and NPA) through the use of battery-operated suction machines for NPA that are suitable to rural areas and an optimization of stool processing without centrifugation allowing stool Xpert testing at lower level health care facilities. This microbiological diagnosis strategy is integrated in a “package of diagnostic approaches”, including the use of standardized/simplified CXR interpretation with quality-assured radiography at district hospital level, capacity building for screening and clinical diagnosis, and the improvement of tuberculosis diagnosis in high-risk children (HIV-infected children, malnourished children, and children with severe pneumonia).

The TB-Speed project will complement efforts made by other consortia or organizations to strengthen NTPs, and to provide the national health authorities and researchers from participating countries with innovative tools and relevant evidence to document and assess the feasibility, acceptability, efficacy, and cost-effectiveness of the new approaches to better integrate childhood tuberculosis screening and

diagnosis across the health system pyramid. The project has 6 outputs which will collectively contribute to the achievement of its goal:

Output 1 is an operational research that will implement and evaluate decentralized childhood tuberculosis diagnostic approaches at district health systems level. This research implemented with the support from National Tuberculosis Programs (NTPs) will use an innovative diagnostic approach based on Xpert MTB/RIF (using the newly developed Xpert Ultra and battery-operated GeneXpert Omni platform) performed on a combination of easy to collect samples (NPA and stool), standardized symptoms screening and chest radiograph interpretation.

Output 2 is a pragmatic cluster randomized clinical trial that will evaluate the impact on mortality and case detection of adding systematic early detection of tuberculosis by Xpert MTB/RIF Ultra performed on NPA and stools to the WHO recommended standard of care for children with severe pneumonia.

Output 3 is a diagnostic validation study for diagnostic tools and algorithms in highly-vulnerable groups with presumptive tuberculosis, specifically HIV-infected and severely malnourished children. This will be a diagnostic cohort study enrolling HIV-infected children and hospitalized severely malnourished children. The study will include an external validation of the PAANTHER algorithm IV-infected children with presumptive tuberculosis and the evaluation of several diagnostic tests in hospitalized severely malnourished children, that could result in the development of a score/algorithm for tuberculosis diagnosis in this population.

Output 4 will consist in the identification of optimized, suitable, and affordable specimen processing and collection methods for childhood tuberculosis diagnosis in resource limited countries. This will involve microbiological and technological optimization work to identify and test simple and affordable specimen processing and collection methods for childhood tuberculosis diagnosis that can be deployed at low health care level in resource-limited countries.

Output 5 will evaluate the cost-effectiveness of the proposed diagnostic approaches and model their market impact to build evidence for public health decision-making.

At last, output 6 will contribute from the start of the project to the dissemination, communication and stakeholders' engagement. This includes communication on the project progresses, advocacy towards the relevant stakeholders, and dissemination of results to support future scaling-up.

The TB Speed project will be conducted in 6 African countries including Sierra Leone, Cote d'Ivoire, Cameroon, Uganda, Zambia, and Mozambique and one South-East Asian country, Cambodia. It includes sites and partners involved in the PAANTHER network and therefore prolongs efforts initiated in the ANRS 12229 PAANTHER 01 study. It is implemented under the leadership of the University of Bordeaux in coordination with a consortium of 7 organizations or institutions including Institut de Recherche pour le Développement in France and Cameroun and MUJHU for Makerere University & Johns Hopkins University collaboration in Uganda, which are co-coordinating the project on scientific aspects with the University of Bordeaux ; the Solthis NGO in Sierra Leone; the PACCI program in Cote d'Ivoire; Institut Pasteur in Cambodia; Instituto Nacional de Saude in Mozambique, and the University of Zambia. I will act both as the overall project director for the University of Bordeaux and coordinating investigator of the overall project or scientific aspects, together with Dr. Maryline Bonnet, from IRD, and Dr. Eric Wobudeya, from Mulago Hospital / Makerere University John Hopkins University collaboration.

9 Conclusion

The ANRS 12229 PAANTHER 01 study, conducted in four resource-limited countries with moderate to very high tuberculosis incidence, enrolled more than 400 HIV-infected children with a suspicion of tuberculosis, one of the major opportunistic infections and the largest cause of death in children and adults living with HIV/AIDS.

Since the study was initiated, there has been major progress made in ensuring access to ART worldwide for children and advances in implementing measures to prevent Mother-To-Child Transmission (MTCT) of HIV. In countries like Cambodia, we have seen the number of children enrolling in HIV cohorts decrease. As in adult, we are still collectively half way to achieving full ART coverage in children worldwide. Furthermore in children as in adults, very severe patients keep entering the health system, being either ART-naïve or having been previously treated for HIV. Access to viral load measurements remains scarce in HIV programs in resource limited settings as is access to 2nd line ART. As a consequence, patients stay on failing ART regimen for long periods of time, progressing from initial virological failure, to immunological, and later clinical failure. They may present with clinical deterioration and presumptive tuberculosis, as did 125 children, enrolled in the PAANTHER 01 study, which had been on ART for more than 6 months.

With two major solutions to enable easier sample collection for Xpert testing and a new algorithm findings from this research could contribute to significant improvement in tuberculosis diagnosis in HIV-infected children. As all algorithms, ours needs to be refined and validated externally to be recommended in a final version, adapted to the field. However, these are significant steps in moving forward rapid and decentralized diagnosis of tuberculosis in HIV-infected children. Challenges remain with the use of diagnostic tools such as CXR, which would benefit from the development and validation of an automated computer-aided detection system for radiographic lesions in children (271). There are many pending research questions around tuberculosis diagnosis and management in HIV-infected children, in a challenging scientific environment due to methodological challenges, as highlighted in our work on reference standards and CXR.

The tuberculosis pandemic itself also has changed in participating countries. Incidences have decreased in some countries such as Cambodia where global incidence is now estimated to 380 cases by 100,000, which has been attributed to the good performances of its national tuberculosis program (278). Tuberculosis in children nevertheless remains a major challenge. In Cameroon, due to improved surveillance system however, the incidence has been re-evaluated to 212 cases per 100,000 population, higher than the 2010 estimates. Diagnostic practices remain very poor in low-resources countries and very poorly decentralized (97). Due to major diagnostic challenges, access to treatment remains limited which explains the greatest share of mortality of this curable disease (5, 10). Building on progress from the study and tools tested in the PAANTHER 01 study, the TB-Speed project, which officially started on October 1, 2017, aims to not only validate the algorithm in HIV-infected children, but also to broaden the question of access to diagnostics for childhood tuberculosis generally, including those presenting with symptoms at community level and in the peripheral district health system, and other vulnerable children, e.g. those with severe pneumonia (123) or those with severe acute malnutrition (279). By improving tuberculosis diagnosis in children and thus contributing to increased case detection and access to treatment, TB-Speed seeks to contribute to the reduction of tuberculosis-related mortality in children, in line with the United Nations Sustainable Development Goals.

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Appendices

Appendix 1: Supplementary appendix to the publication “Mortality and its determinants in ART-naïve HIV-infected children with a suspicion of tuberculosis, an observational cohort study”

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2. Study sites

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Cambodia

National Pediatric Hospital, Phnom Penh,

Angkor Hospital for Children, Siem Reap.

Cameroon

Centre Hospitalier de la Caisse d'Essos, Yaounde

Centre Mère et Enfant de la Fondation Chantal Biya, Yaounde

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Pham Ngoc Thach Hospital, Pediatric Department, Ho Chi Minh City

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3. Ethics committees, institutional review boards, and national authorities

Burkina Faso

Ethics Committee for Research in Health

Cambodia

National Ethics Comity for Health and Research, Phnom Penh, Cambodia

Cameroon

National Ethics Committee

Ministry of Public Health, Division of Health Operations Research

Vietnam

Pham Ngoc Thach Hospital Institutional Review Board

Ho Chi Minh City Department of Health

Ho Chi Minh City People's Committee

4. ANRS 12229 PAANTER 01 Study protocol:

Accessible at: http://www.pasteur-kh.org/wp-content/uploads/2016/01/ANRS-12-229_PAANTHER_01_KH_Protocol_Version-3.0_English_2013_08_08_online.pdf

5. Additional methods

Table S1. Updated Clinical Case Definition for Classification of Intrathoracic Tuberculosis in Children (adapted from S. Graham et al, CID, 2015)

Case definition	Refined criteria
Confirmed tuberculosis	Bacteriological confirmation obtained (Mycobacterium tuberculosis confirmed by culture or Xpert MTB/RIF assay from at least 1 respiratory specimen)
Unconfirmed tuberculosis	Bacteriological confirmation NOT obtained AND at least 2 of the following: <ul style="list-style-type: none">• Symptoms/signs suggestive of tuberculosis (as defined)• Chest radiograph consistent with tuberculosis*• Close tuberculosis exposure or immunologic evidence of M. tuberculosis infection• Positive response to tuberculosis treatment (requires documented positive clinical response on tuberculosis treatment—no time duration specified) AND no spontaneous improvement of symptoms in the absence of antituberculosis treatment
Unlikely tuberculosis	Bacteriological confirmation NOT obtained AND Criteria for “unconfirmed tuberculosis” NOT met (including spontaneous improvement of symptoms in the absence of antituberculosis treatment)

*CXR were considered consistent with tuberculosis if reviewers agreed on the presence and location (right/left, and upper or median/lower lobe for alveolar opacities) of ≥ 1 lesion among the following: alveolar opacity, bronchial compression, excavation, ghon focus, gibbus, miliary, nodular infiltrates, paratracheal nodes, peri-hilar nodes, pleural effusion, tracheal compression (as suggested by Graham et al. 2012).

6. Additional results

Table S2. Factors associated with elevated aspartate aminotransferase (AST) at baseline

	Children		AST (U/L)				Univariate analysis			Multivariate analysis		
	n	n	median, Q1, Q3				Odds ratio (95%CI)		p value	Odds ratio (95%CI)		p value
Sex									0.9303			
Male	137	131	38	19	69	1						
Female	129	126	33	20	51	0.98	0.665	1.452				
Age									<.0001			<.0001
<2	61	55	50	32	72	1				1		
[2 - 5[46	45	36	20	62	0.37	0.192	0.705		0.339	0.152	0.756
[5 - 10[114	113	29	16	51	0.22	0.132	0.380		0.187	0.089	0.390
≥10	45	44	26	17	45	0.17	0.090	0.323		0.129	0.051	0.325
Country									<.0001			0.0250
Burkina Faso	25	25	16	10	22	1				1		
Cambodia	52	51	21	15	45	0.99	0.458	2.142		1.390	0.494	3.917
Cameroon	107	101	40	27	70	4.05	1.959	8.376		1.928	0.727	5.117
Vietnam	82	80	44	23	79	4.75	2.284	9.894		3.503	1.264	9.709
Weight for age Z score (SD)									<.0001			
≥-2	82	80	29	20	46	1						
[-3 to -2[89	86	24	15	66	1.29	0.780	2.137				
[-4 to -3[46	45	44	23	60	2.33	1.282	4.222				
<-4	48	45	50	35	73	3.63	2.006	6.555				
Immune depression									<.0001			0.0001
Not significant	40	40	22	15	41	1				1		
Mild and advanced	20	19	29	21	65	3.08	1.235	7.661		1.312	0.389	4.428
Severe	60	59	34	20	66	6.59	3.281	13.243		2.784	1.136	6.821
Very severe (<10%)	134	134	43	22	70	9.02	4.814	16.890		5.319	2.404	11.771
HIV RNA (Log10 copies/mL)												
<5.5	76	75	30	19	51							
[5.0 – 5.5[62	62	29	17	56							
[5.5 – 6.0[57	56	44	24	73							
≥ 6.5	53	52	45	28	66							
Nontuberculous mycobacteria in culture									0.2210			
No	233	224	37	20	63	1						
Yes	33	33	22	16	67	1.45	0.798	2.647				
Culture confirmed tuberculosis									0.0238			
No	229	220	35	20	64	1						
Yes	37	37	31	19	64	1.95	1.093	3.484				
Tuberculosis reference diagnosis									0.3017			
Confirmed tuberculosis						1						
Unconfirmed tuberculosis						1.59	0.878	2.873				
Unlikely tuberculosis						1.09	0.709	1.661				
Hemoglobin (g/dL)									<.0001			0.0115
≥11.0	49	49	23	17	65	1				1		
[9.0 – 11.0[107	107	33	20	52	2.64	1.542	4.525		2.109	1.051	4.231
[7.0 – 9.0[72	70	41	24	67	3.84	2.157	6.847		1.651	0.762	3.575

<7.0	31	31	40	19	66	6.57	3.169	13.609		5.012	1.863	13.484
Platelets									<.0001			
<50 000	9	9	20	15	83	3.62	1.149	11.390				
[50 000 - 150 000]	26	25	64	30	113	3.95	2.007	7.772				
>150 0000	224	223	32	20	52	1						
Chest radiograph anomalies									0.0690			
No	27	27	29	19	67	1						
Yes	224	220	34	20	56	1.79	0.956	3.333				
Chest radiograph consistent with tuberculosis									0.1649			
Yes	176	173	36	20	60	1						
No	69	68	29	17	52	0.74	0.485	1.132				
Miliary									0.4486			
Yes	17	17	29	16	43	1.45	0.555	3.780				
No	233	229	35	20	64	1						
Alveolar opacity									0.1532			
Yes	143	141	34	20	69	0.75	0.500	1.115				
No	107	105	31	19	49	1						
Abdominal distension									<.0001			
Yes	62	57	44	24	66	1						
No	200	196	31	19	56	0.349	0.211	0.577				
Hepatomegaly									<.0001			
Yes	113	106	43	23	66	1						
No	146	144	29	18	53	0.373	0.247	0.562				
Jaundice									0.9837			
Yes	2	1	219	219	219	1						
No	258	250	34	20	64	<0.001	<0.001	>999.99				
Abdominal mass									0.0116			
Yes	16	16	51	39	91	1						
No	247	238	32	19	60	0.327	0.137	0.779				
Splenomegaly									0.0001			
Yes	64	60	42	24	68	1						
No	197	192	33	19	56	0.375	0.229	0.615				
Abdominal tenderness									0.0432			
Yes	36	35	40	24	73	1						
No	228	220	32	19	56	0.548	0.306	0.982				
Ascites on ultrasonography									0.7270			
Yes	12	12	34	17	45	1						
No	235	233	33	19	60	0.832	0.295	2.343				
Hepatomegaly on ultrasonography									<.0001			0.0033
Yes	67	67	40	23	83	1				1		
No	180	178	31	18	49	0.400	0.252	0.633		0.391	0.209	0.732
Abdominal lymph node on ultrasonography									0.5879			
Yes	66	66	24	15	45	1						
No	181	179	37	20	66	1.137	0.715	1.806				
Abdominal ultrasonography									0.0474			
Normal	119	118	33	19	51	1						
Abnormal	128	127	33	19	65	1.509	1.005	2.266				
Splenomegaly on ultrasonography									0.7028			
Yes	21	21	40	19	60	1						

No	226	224	32	19	56	1.164	0.533	2.542
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Appendix 2. Editorial Commentary: Improving Children's Access to New Tuberculosis Diagnostic Tools Starts With the Collection of Appropriate Specimens

This editorial supplementary by Anne Detjen and Elisabetta Walters was published in Clinical Infectious Diseases journal on May 1, 2016, together with our publication on use of Xpert MTB/RIF with alternative specimens.

Appendix 3 Supplementary appendix to the publication “Performance of Xpert MTB/RIF and alternative specimen collection methods for the diagnosis of tuberculosis in HIV-infected children”

1. List of members of the ANRS 12229 PAANTHER study group (Cf. Appendix 1)

2. Study design - bacteriological specimen collection schedule

Table S1. Bacteriological specimen collection methods

Age	Day 1	Day 2	Day 3
< 4 years	Gastric aspirate ^a	Gastric aspirate ^a	Gastric aspirate ^a
4 to 9 years	Gastric aspirate ^a	Gastric aspirate ^a	String test
≥ 10 years	Sputum sample ^{a,b}	Sputum sample ^a	String test
All	Nasopharyngeal aspirate Stool sample ^c		

^a standard samples, ^b doubled on the same day, ^c collected at day2 or 3 if not done at day 1

3. Specimen collection and Xpert procedures

3.1. String test

The string test was performed with the Pediatric Entero-test® (HDC Corporation, Milpitas, California, USA), an FDA-approved device for collection of gastric contents in children for detection of parasites and bacteria (*Helicobacter Pylori*). It is a device consisting of a 90 cm nylon string coiled into a gelatin capsule (the size of amoxicillin 250 mg capsule).

On the day of the string test the child should have ideally fasted overnight. The procedure was performed in a child sitting by himself or on his family member/guardian's lap, after re-explaining to the child and the accompanying person the reason for sample collection and the steps of the procedure.

First, 20/30 cm of the thin non-absorbent part of the string was unraveled, and the child was asked to open his/her mouth and stick out his/her tongue. The capsule was placed by the nurse on the back of the child's tongue with the finger (or tongue depressor), with 10/20 cm of nylon line coiled on the tongue, the extremity of the string held in firmly hand, and the child was given a big glass of water (200 mL) to swallow the capsule. If the child had nausea right after swallowing the capsule, s/he was asked to swallow saliva or given more water to drink. Once the capsule was swallowed, the upper extremity of the string was attached to the child's cheek with the adhesive tape and left in place for 2 hours. During the "down-time" the child was occupied and distracted with quiet activities (puzzles, drawing, quiet

games) to minimize irritation and prevent inadvertent or deliberate string removal. In the case of young children, parents or guardian were asked to distract the child from the string. The child was instructed to swallow saliva normally during the test and allowed to drink small amounts of water if needed. After two hours, the string was removed by tilting the child's head back and gently pulling the line upward and out for 5 seconds. The thick part of the string was then cut with scissors from the thin nylon extremity (~20/30 cm kept in mouth) and deposited into a 50 mL falcon tube containing 3 mL 0.9% saline serum. After cleaning of the Falcon tube with alcohol to prevent cross-infection, and appropriate labeling, the specimens were transported to the laboratory within 4 hours.

3.2. Nasopharyngeal aspirates

Nasopharyngeal aspirates consisted in the collection of 2-5 ml of throat contents through a catheter tube connected to a mucus aspirator. Nasopharyngeal aspirate was done for all children, early in the morning after overnight fasting and after gastric aspirates for children up to 10 years old or sputum collection for children older than 10 years.

The procedure was performed in a child in supine position on his/her back or side, or sitting on his family member/guardian's lap, after re-explaining to the child and the accompanying person the reason for sample collection and the steps of the procedure. In order to avoid child injury due to movement, young children were wrapped in a piece of cloth, and an assistant nurse asked to hold the child's head throughout procedure.

After connecting a mucus extractor to the suction pump and catheter, the suction pressure was adjusted. The pressure and catheter size were recommended based on the child's age as follows: in children aged < 1 year, 8 CH catheter with 80-100 mmHg (0.10 bar) suction pressure; in children aged 1 to 10 years, 8 CH catheter with 100-120 mmHg (0.15 bar) suction pressure; and in children aged >10 years, 10 CH catheter with 120-150 mmHg (0.20 bar) suction pressure. After measuring the length of tube necessary to reach the posterior pharynx, equal to the distance between the tip of the nose and the external opening of the ear, the catheter was inserted into the child's nose, without applying suction, along the nostril floor to the posterior pharyngeal wall.

This usually induced cough and sputum expectoration that could be aspirated by applying suction and slowly withdrawing catheter, using a rotating movement, without pushing the catheter forward while aspirating to reduce the risk of local trauma. The catheter should remain in nasopharynx for a minimal period of time, not to exceed 10 seconds.

This procedure should aspirate 2 to 5 ml of secretions. If the volume was not reached by the first aspiration, the procedure was repeated with nasopharyngeal lavage by inserting 5ml of normal saline in nostril and repeated in the other nostril. This procedure was not repeated more than twice. After recapping and cleaning of the specimen container with alcohol/chlorhexidine to prevent cross-infection, and appropriate labeling, the specimens were transported to the laboratory within 4 hours.

The entire procedure was performed under peripheral oxygen saturation monitoring with an oximeter.

3.3. Preparation of stool samples for Xpert MTB/RIF

Stool samples were prepared for Xpert MTB/RIF testing by emulsification of 0.5 g of material in Sheather's solution, filtering through funnel gauze and centrifugation.

Sheather's solution was previously prepared by dissolving 454 g of sucrose in 355 mL of distilled water over low-heat on a stove. After autoclaving for 15 min at 110°C, 10 mL aliquots of this solution were prepared in sterile 15 mL Falcon tube and kept at 4° C to prevent mold contamination.

Stool samples were processed by adding 10 mL of the 50% Sheather's solution to 0.5 g of fresh stool specimen or frozen stool specimen thawed at room temperature into a 15 mL Falcon tube, emulsifying stool manually with two wooden sticks, and vortexing for 30 seconds. The emulsion obtained was poured through funnel-gauze into a new 15 mL Falcon tube, and the centrifuged at 100 x g for 1 minute (no brake). After careful removal of the tube from the centrifuge to avoid disturbing the suspension, 0.5 mL of suspension was retrieved from the top of the specimen and added to 1.8 mL of Xpert MTB/RIF Sample Reagent, shaken vigorously 10 - 20 times, and incubated for 15 minutes at room temperature. After 5 to 10 minutes of incubation, the specimen was shaken again vigorously 10 to 20 times. The specimen obtained was then directly tested with the Xpert MTB/RIF.

4. Microbiology methods

Table S2. Microbiology methods in the different countries participating to the study

Country	Mycobacterial culture	Mycobacterium tuberculosis complex Identification	Drug susceptibility testing/resistance determination methods
Burkina Faso	Lowenstein Jensen	Niacin test TB Ag MPT64	Proportion method (on Lowenstein Jensen)
Cambodia	Lowenstein Jensen and Bactec MGIT	Gen-Probe AccuProbe <i>Mycobacterium tuberculosis</i> complex culture identification test	Bactec MGIT S.I.R.E.
Cameroon	Lowenstein Jensen and Bactec MGIT	SD MTP64 Ag Kit	Bactec MGIT S.I.R.E
Vietnam	Bactec MGIT	Niacin test	Proportion method (on Lowenstein Jensen)

5. Additional results

Table S3. Characteristics defining the TB reference diagnostic

Characteristics defining the TB reference diagnostic	All (n=272)	Confirmed TB (n=29)	Probable TB (n=116)	Possible TB (n=100)	TB unlikely and not TB (n=27)
Microbiological confirmation ^a	29 (10.7)	29 (100)	0	0	0
Any sign and symptom suggestive of TB	267 (98.2)	28 (96.6)	116 (100)	100 (100)	23 (85.2)
Cough lasting more than two weeks in anamnesis	229 (84.2)	21 (72.4)	98 (84.5)	90 (90.0)	20 (74.1)
Weight loss/failure to thrive (weight/age or weight/height ≤ -2)	193 (71.0)	19 (65.5)	92 (79.3)	63 (63.0)	19 (70.4)
Persistent ^b unexplained fever	147 (54.0)	20 (69.0)	65 (56.0)	55 (55.0)	7 (25.9)
Persistent ^b , unexplained lethargy or reduced playfulness	102 (37.5)	15 (51.7)	36 (30.2)	38 (38.0)	14 (51.9)
Additional signs and symptoms suggestive of TB in infants 0–60 days	0	0	0	0	0
Chest X-ray consistent with intra-thoracic TB disease	176 (64.7)	17 (58.6)	116 (100)	43 (43.0)	0
History of exposure to <i>M. tuberculosis</i>	29 (10.7)	5 (17.2)	14 (12.1)	6 (6.0)	4 (14.8)
Immunological evidence of <i>M. tuberculosis</i> infection (TST ≥ 5 mm)	25 (9.2)	6 (20.7)	14 (12.1)	5 (5.0)	0
Response to anti-tuberculosis treatment at 2 months (n=143)	126 (46.3)	17 (58.6)	82 (70.7)	26 (26.0)	1 (3.8)
Alternative diagnosis given by treating clinician (n=268)	141 (51.8)	4 (13.8)	42 (36.2)	74 (74.0)	21 (77.8)

Abbreviations: TB, tuberculosis; TST, tuberculin skin test. ^a at least 1 positive culture with *Mycobacterium tuberculosis* speciation from sputum, nasopharyngeal aspirates, gastric aspirates, or string tests collected. ^b lasting more than 2 weeks.

Table S4. Bacteriological specimen collection methods performed by age

Age		Samples planned per protocol performed				
< 4 years	Gastric aspirate 1	Gastric aspirate 2	Gastric aspirate 3		Nasopharyngeal aspirate	Stool sample
	(N=67)	67 (100%)	65 (97.0%)	63 (94.0)	67 (100.0)	64 (95.5)
4 to 9 years	Gastric aspirate 1	Gastric aspirate 2		String test	Nasopharyngeal aspirate	Stool sample
	(N=141)	138 (97.9%)	136 (96.4%)	103 (73.0)	138 (97.9)	139 (98.6)
≥ 10 years	Sputum sample 1	Sputum sample 2	Sputum sample 3	String test	Nasopharyngeal aspirate	Stool sample
	(N=64)	55 (86.0%)	52 (81.2%)	46 (71.9)	59 (92.2)	63 (98.5)

Table S5. Characteristics of children with positive Xpert and negative TB culture

	Sex	Country	Weight for Age Z score	On ART	ART duration	CD4 %	VL (log)	Hb count (g/L)	Cough > 2 weeks	Weight loss/failure to thrive	Persistent ever	Persistent lethargy or reduced playfulness	History of exposure to MTB	Immunologic evidence of MTB infection (TST ≥ 5 mm)	Response to anti-TB treatment	Chest X-ray consistent with intra-thoracic TB disease	Vital status at end of study
1	M	BF	0	yes	6.8	27.5	4.3	11.7	Yes	no	no	no	no	no	no	no	reached M6
2	F	BF	-2.1	yes	5.0	14.5	5.0	11.4	Yes	yes	no	no	no	no	no	no	reached M6
3	M	BF	-1.2	yes	0.3	25.0	3.2	10.7	Yes	no	no	yes	no	no	yes	yes	reached M6
4	F	BF	-2.5	no	-	4.3	6.0	6.5	Yes	yes	yes	yes	no	no	yes	yes	reached M6
5	M	KH	-3.8	no	-	1.0	5.8	9.9	Yes	yes	yes	yes	yes	no	yes	yes	reached M6
6	F	VN	-3.7	no	-	5.3	7.4	6.7	Yes	yes	yes	yes	no	no	no	yes	death at 12 days

ART, antiretroviral therapy; VL, viral load; Hb, hemoglobin; MTB, mycobacterium tuberculosis; TST, tuberculin skin test; TB, tuberculosis; BF, Burkina Faso;

KH, Cambodia; VN, Vietnam; M6, follow-up visit at 6 month (end of study).

Appendix 4 - Supplementary appendix to the publication: “A diagnostic prediction score and algorithm for tuberculosis treatment decision in HIV-infected children with a suspicion of tuberculosis.”

SUPPLEMENTARY METHODS

Procedures and definitions

Cough patterns graphic illustration shown to parents, adapted from Marais et al, Archives of Disease in Childhood, 2005 (280).

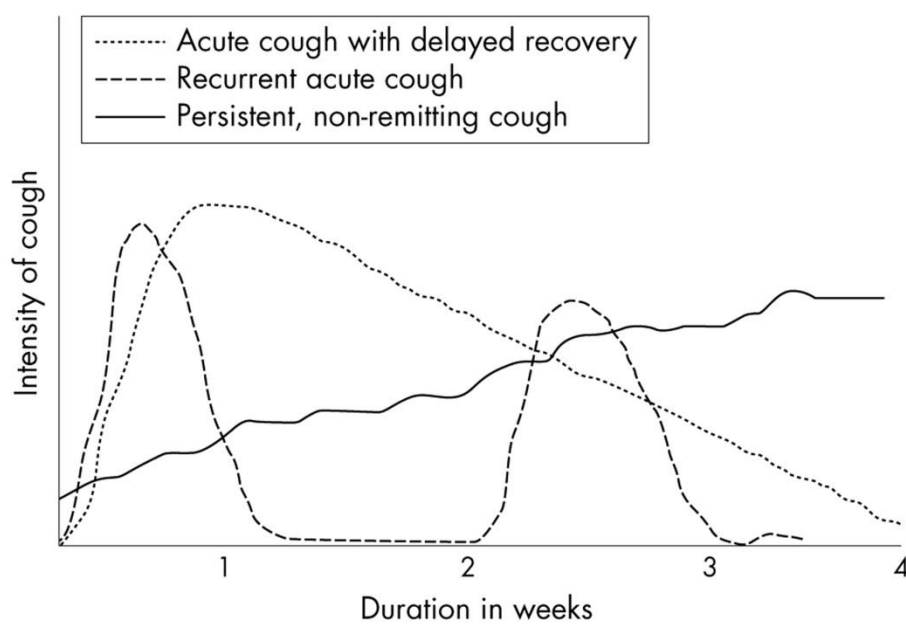


Table S1. Updated Clinical Case Definition for Classification of Intrathoracic Tuberculosis in Children (adapted from S. Graham et al, CID, 2015)

Case definition	Refined criteria
Confirmed tuberculosis	Bacteriological confirmation obtained (Mycobacterium tuberculosis confirmed by culture or Xpert MTB/RIF assay from at least 1 respiratory specimen)
Unconfirmed tuberculosis	Bacteriological confirmation NOT obtained AND at least 2 of the following: <ul style="list-style-type: none"> • Symptoms/signs suggestive of tuberculosis (as defined) • Chest radiograph consistent with tuberculosis* • Close tuberculosis exposure or immunologic evidence of M. tuberculosis infection • Positive response to tuberculosis treatment (requires documented positive clinical response on tuberculosis treatment—no time duration specified) AND no spontaneous improvement of symptoms in the absence of antituberculosis treatment
Unlikely tuberculosis	Bacteriological confirmation NOT obtained AND Criteria for “unconfirmed tuberculosis” NOT met (including spontaneous improvement of symptoms in the absence of antituberculosis treatment)

*CXR were considered consistent with tuberculosis if reviewers agreed on the presence and location (right/left) of ≥ 1 lesion among the following: alveolar opacity, bronchial compression, excavation, ghon focus, gibbus, miliary, nodular infiltrates, paratracheal nodes, peri-hilar nodes, pleural effusion, tracheal compression (as suggested by Graham et al. 2012).

Table S2. Age-defined tachycardia and tachypnea

Age (years)	Heart rate defining tachycardia	Respiratory rate defining tachypnea
≤ 1	>160	>60
[1 – 2]	>150	>40
[2 - 5]	>140	>40
>5	>120	>40

Statistical analysis

We included as candidate predictors characteristics used in previous childhood tuberculosis scoring systems or characteristics known to be associated with the diagnosis of tuberculosis in children as well as in adults with HIV-infection from the literature. This included the following predictors: presence of abdominal lymphadenopathies, as well as ascites, hepatomegaly, and splenomegaly on the ultrasonography (281, 282), reported fatigue or loss of playfulness, unremitting persistent cough for more than two weeks (100), contact with a tuberculous index case (ref), weight loss, anorexia, night sweats, hepatomegaly and or splenomegaly, chronic or intermittent fever, history of exposure to infectious tuberculosis, signs of fluid in chest (dull to percussion/reduced breath sounds), chronic cough >30 days, fever > 14 days, enlarged lymph nodes, abdominal swelling with or without palpable lump, swelling of bone or joint (282), adenopathy, consolidation, cavitation (283).

Supplementary results

Table S3. Characteristics of cases (culture-confirmed tuberculosis) and controls (unlikely tuberculosis alive at M6 and not-treated)

	Controls (N=153)		Cases (N=45)	
	N	% or median (IQR)	n	% or median (IQR)
Age	153	8.0 (3.5 – 10.3)	45	7.8 (5.6 – 9.9)
Sex. Male	66	43.1	25	55.6
Country				
Burkina Faso	33	21.6	6	13.3
Cameroon	45	29.4	21	46.7
Cambodia	34	22.2	3	6.7
Vietnam	41	26.8	15	33.3
Underweight (weight for age Z score <-2)	81	54.4	26	60.5
Weight for age Z score	149	-2.4 (-2.9 to -1.4)	43	-2.2 (-3.3 to -1.4)
Lansky play performance score	153	100 (80 – 100)	44	80 (60 – 90)
CD4 absolute count	142	664 (114 – 1096)	45	155 (28 – 819)
CD4 %age	139	16.8 (4.9 – 26.8)	45	10.2 (2.6 – 21.3)
Immune depression [†]				
Not significant	53	38.1	12	26.7
Mild and advanced	11	7.9	2	4.4
Severe	28	20.1	11	24.4
Very severe	47	33.8	20	44.4
On ART at inclusion	73	47.7	9	20.0
Hemoglobin (g/dL)	152	10.5 (9.1 – 11.8)	45	8.3 (6.6 – 9.7)
TB history				
Yes	11	7.2	5	11.4
No	139	90.8	39	88.6
Unknown	3	2.0		
CXR consistant with TB				
No	77	52.0	10	25.6
Yes	71	48.0	29	74.4
Tuberculin skin test				
Negative	128	92.1	31	79.5
Positive	11	7.9	8	20.5
NTM in culture				
No	138	90.2	40	88.9
Yes	15	9.8	5	11.1
Smear or Xpert + at baseline				
Negative	153	100.0	12	26.7
Positive	0	0.0	33	73.3
Smear result				
Negative	153	100.0	23	51.1
Positive	0	0.0	22	48.9
Xpert result				
Negative	153	100.0	11	24.4
Positive	0	0.0	34	75.6
TB treatment				
No	153	100.0	3	6.7
Yes	0	0.0	42	93.3

Table S4. Tuberculosis tests and predictors, case control sub-study

	n	N	Se	IC95 %		n	Not TB	Sp		
Cough										
In past 24 hours	35	44	79.5	67.6	91.5	15	153	9.8	5.1	14.5
Any in past 4 wks	38	44	86.4	76.2	96.5	11	153	7.2	3.1	11.3
Lasting >2 wks	34	44	77.3	64.9	89.7	29	153	19.0	12.7	25.2
Lasting >3 wks	25	44	56.8	42.2	71.5	63	153	41.2	33.4	49.0
Lasting >4 wks	23	44	52.3	37.5	67.0	78	153	51.0	43.1	58.9
Unremitting cough	15	44	34.1	20.1	48.1	127	153	83.0	77.1	89.0
Fever										
In past 24 hours	36	44	81.8	70.4	93.2	92	153	60.1	52.4	67.9
Any in past 4 wks	36	38	94.7	87.6	101.8	41	102	40.2	30.7	49.7
Lasting >2 wks	31	44	70.5	57.0	83.9	101	153	66.0	58.5	73.5
Lasting >3 wks	16	44	36.4	22.1	50.6	125	153	81.7	75.6	87.8
Lasting >4 wks	15	44	34.1	20.1	48.1	133	153	86.9	81.6	92.3
Abdominal pain >2 wks	12	39	30.8	16.3	45.3	110	130	84.6	78.4	90.8
Loss of appetite in past 4 wks	26	44	59.1	44.6	73.6	93	153	60.8	53.0	68.5
Loss of appetite lasting >2 wks	6	44	13.6	3.5	23.8	136	153	88.9	83.9	93.9
Chest pain in past 4 wks	8	36	22.2	8.6	35.8	95	123	77.2	69.8	84.6
Diarrhea in past 4 wks	9	44	20.5	8.5	32.4	116	153	75.8	69.0	82.6
Dyspnea in past 4 wks	7	43	16.3	5.2	27.3	127	152	83.6	77.7	89.4
Fatigue in past 4 wks	28	43	65.1	50.9	79.4	98	153	64.1	56.4	71.7
Fatigue lasting >2 wks	21	43	48.8	33.9	63.8	116	153	75.8	69.0	82.6
Headaches lasting >2 wks	5	36	13.9	2.6	25.2	116	121	95.9	92.3	99.4
Hemoptysis in past 4 wks	1	44	2.3	-2.1	6.7	148	152	97.4	94.8	99.9
Loss of playfulness in past 4 wks	25	43	58.1	43.4	72.9	106	153	69.3	62.0	76.6
Sleep disorders in past 4 wks	11	44	25.0	12.2	37.8	131	152	86.2	80.7	91.7
Drenching night sweats in past 4 wks	11	44	25.0	12.2	37.8	106	153	69.3	62.0	76.6
Vomitting in past 4 wks	5	44	11.4	2.0	20.7	118	152	77.6	71.0	84.3
Weight loss in past 4 wks	39	44	88.6	79.3	98.0	74	153	48.4	40.4	56.3
Temperature > 37.8°C	30	44	68.2	54.4	81.9	123	151	81.5	75.3	87.7
Tachycardia	20	44	45.5	30.7	60.2	139	150	92.7	88.5	96.8
Tachypnea	17	40	42.5	27.2	57.8	100	142	70.4	62.9	77.9
Chest wall indrawing	6	44	13.6	3.5	23.8	146	152	96.1	93.0	99.1
Abnormal lung sounds auscultation	26	44	59.1	44.6	73.6	68	153	44.4	36.6	52.3
Reduced breath sounds	11	44	25.0	12.2	37.8	122	152	80.3	73.9	86.6
Crackles	22	44	50.0	35.2	64.8	85	153	55.6	47.7	63.4
Rhonchus	11	44	25.0	12.2	37.8	134	153	87.6	82.4	92.8
Wheezing	4	44	9.1	0.6	17.6	141	153	92.2	87.9	96.4
Dullness on percussion	10	44	22.7	10.3	35.1	136	152	89.5	84.6	94.4
Any lymph node	20	44	45.5	30.7	60.2	104	153	68.0	60.6	75.4
Cervical lymph node	19	44	43.2	28.5	57.8	109	151	72.2	65.0	79.3
Hepatomegaly	22	43	51.2	36.2	66.1	118	150	78.7	72.1	85.2
Splenomegaly	18	43	41.9	27.1	56.6	130	149	87.2	81.9	92.6

Abdominal distension	11	43	25.6	12.5	38.6	142	153	92.8	88.7	96.9
Abdominal tenderness	10	44	22.7	10.3	35.1	141	153	92.2	87.9	96.4
Abdominal mass	6	44	13.6	3.5	23.8	147	153	96.1	93.0	99.2
QFT										
Without indeterminate	25	31	80.6	66.7	94.6	111	124	89.5	84.1	94.9
Indeterminate = negative	25	42	59.5	44.7	74.4	137	150	91.3	86.8	95.8
Contage	7	45	15.6	5.0	26.1	142	153	92.8	88.7	96.9
Contact with smear+ TB case	7	45	15.6	5.0	26.1	150	153	98.0	95.8	100.2
Ultrasonography										
Abdominal lymph nodes	21	42	50.0	34.9	65.1	125	146	85.6	79.9	91.3
Chest radiograph										
Ghon focus	0	42	0.0	0.0	0.0	152	152	100	100	100
Excavation	3	42	7.1	-0.6	14.9	151	151	100	100	100
Miliary	5	42	11.9	2.1	21.7	147	152	96.7	93.9	99.5
Paratracheal nodes	2	42	4.8	-1.7	11.2	148	152	97.4	94.8	99.9
Tracheal compression	2	42	4.8	-1.7	11.2	152	152	100	100	100
Peri-hilar lymph nodes	5	42	11.9	2.1	21.7	133	152	87.5	82.2	92.8
Nodular opacities	5	42	11.9	2.1	21.7	140	152	92.1	87.8	96.4
Alveolar opacity	15	42	35.7	21.2	50.2	118	152	77.6	71.0	84.3
Pleural effusion	3	42	7.1	-0.6	14.9	145	152	95.4	92.1	98.7
Bronchial compression	1	42	2.4	-2.2	7.0	152	152	100	100	100
Gibbus	0	42	0.0	0.0	0.0	151	151	100	100	100

Table S5. Characteristics of children with and without data available on candidate predictors

	Data on candidate predictors available				P- Value
	No (N=105)		Yes (N=333)		
	n	(%) or median (IQR)	n	(%) or median (IQR)	
Age	105	6.10 (1.2 - 9.5)	333	7.4 (3.9 – 9.7)	0.0029
Sex, male	55	(52.4)	165	(49.5)	0.6550
Country					<.0001
Burkina Faso	20	(19.0)	43	(12.9)	
Cameroon	50	(47.6)	75	(22.5)	
Cambodia	9	(8.6)	130	(39.0)	
Vietnam	26	(24.8)	85	(25.5)	
Underweight (weight for age Z score <-2)	68	(70.8)	206	(62.5)	0.1314
Weight for age Z score	96	-2.69 (-4.35 to -2.00)	333	-2.47 (-3.13 to -1.74)	0.0033
Lansky play performance score	99	80 (70 – 100)	333	80 (80 -100)	0.4001
CD4 absolute count	90	471 (36 – 929)	326	463 (53 – 1026)	0.7808
CD4 %age	90	12.9 (4.0 – 21.0)	324	14.4 (3.0 – 25.0)	0.5478
Immune depression [†]					0.0022
Not significant	19	(21.1)	109	(33.6)	
Mild and advanced	8	(8.9)	29	(9.0)	
Severe	30	(33.3)	52	(16.0)	
Very severe	33	(36.7)	134	(41.4)	
On ART at inclusion	33	(31.4)	139	(41.7)	0.0592
Hemoglobin (g/dL)	95	9.5 (7.8 – 10.7)	332	10.3 (8.7 – 11.6)	0.0003
TB history					0.0012
Yes	3	(2.9)	54	(16.2)	
No	98	(94.2)	264	(79.3)	
Unknown	3	(2.9)	15	(4.5)	
CXR consistant with TB					0.4317
No	27	(29.7)	105	(31.5)	
Yes	62	(68.1)	211	(63.4)	
Disagreement	2	(2.2)	17	(5.1)	
Tuberculin skin test					0.2946
Negative	72	(94.7)	285	(91.1)	
Positive	4	(5.3)	28	(8.9)	
Culture confirmed TB					0.4862
No	82	(87.2)	298	(89.8)	
Yes	12	(12.8)	34	(10.2)	
NTM in culture / NTM paper					0.0495
Negative	89	(94.7)	291	(87.7)	
Positive	5	(5.3)	41	(12.3)	
Smear result					0.9838
Negative	88	(93.6)	311	(93.7)	
Positive	6	(6.4)	21	(6.3)	
Xpert result					0.3346
No	82	(87.2)	300	(90.6)	
Yes	12	(12.8)	31	(9.4)	

TB diagnosis Graham 2015					0.1173
Confirmed TB	14	(13.3)	41	(12.3)	
Unconfirmed TB	38	(36.2)	158	(47.4)	
Unlikely TB	53	(50.5)	134	(40.2)	
TB treatment					0.0484
No	56	(53.3)	141	(42.3)	
Yes	49	(46.7)	192	(57.7)	
Time to TB Tx initiation (days)	49	7 (4 – 11)	192	7 (6 – 11)	0.2631
Died					0.0011
No	80	(76.2)	296	(88.9)	
Yes	25	(23.8)	37	(11.1)	

Table S6. Diagnostic prediction models without microbiological confirmation

Predictor	Model 1 with QFT and abdominal ultrasonography				Model 2 with abdominal ultrasonography (no QFT)				Model 3 with QFT (no abdominal ultrasound)				Model 4 (no QFT or abdominal ultrasonography)			
	β^*	OR	(95%CI)	P-Value	β^\dagger	OR	(95%CI)	P-Value	β^\dagger	OR	(95%CI)	P-Value	β^{**}	OR	(95%CI)	P-Value
Fever lasting more than 2 weeks				<0.0001				<0.0001				<0.0001				<0.0001
No		1				1				1				1		
Yes	1.176	3.24	(1.85 - 5.69)		1.141	3.13	(1.80 - 5.44)		1.0921	2.98	(1.74 - 5.11)		1.069	2.91	(1.71 - 4.96)	
Unremitting cough				0.0823				0.1279				0.0724				0.1070
No		1				1				1				1		
Yes	0.610	1.84	(0.93 - 3.66)		0.528	1.70	(0.86 - 3.35)		0.6066	1.83	(0.95 - 3.56)		0.538	1.71	(0.89 - 3.30)	
Hemoptysis in previous 4 weeks				0.0685				0.0730				0.1236				0.1344
No		1				1				1				1		
Yes	1.623	5.07	(0.88 - 29.07)		1.574	4.83	(0.86 - 26.97)		1.3542	3.87	(0.69 - 21.72)		1.300	3.67	(0.67 - 20.12)	
Weight loss in previous 4 weeks				0.1191				0.0890				0.0425				0.0311
No		1				1								1		
Yes	0.481	1.62	(0.88 - 2.96)		0.518	1.68	(0.92 - 3.05)		0.6056	1.832	(1.02 - 3.29)		0.634	1.89	(1.06 - 3.36)	
Contact with smear + TB case				0.0044				0.0032				0.0107				0.0075
No		1				1				1				1		
Yes	2.440	11.47	(2.14 - 61.35)		2.502	12.20	(2.32 - 64.25)		2.1384	8.49	(1.64 - 43.82)		2.220	9.20	(1.81 - 46.79)	
Tachycardia				0.0617				0.0277				0.0396				0.0143
No		1				1								1		

Yes	0.940	2.56	(0.96 – 6.87)		1.081	2.95	(1.13 - 7.72)		0.9950	2.71	(1.05 - 6.98)		1.165	3.21	(1.26 - 8.15)	
Miliary on CXR				0.0222				0.0175				0.0321				0.0279
No		1				1								1		
Yes	1.773	5.89	(1.29 - 26.94)		1.823	6.19	(1.38 – 27.86)		1.6209	5.06	(1.15 - 22.28)		1.636	5.13	(1.19 - 22.07)	
Alveolar opacities on CXR				0.0004				0.0002				0.0033				0.0017
No		1				1								1		
Yes	1.166	3.21	(1.69 - 6.10)		1.220	3.39	(1.80 – 6.38)		0.9220	2.51	(1.36 - 4.65)		0.962	2.62	(1.43 - 4.78)	
Lymph nodes on CXR				<0.0001				<0.0001				<0.0001				<0.0001
No		1				1								1		
Yes	1.692	5.43	(2.75 - 10.71)		1.571	4.81	(2.48 – 9.34)		1.8505	6.36	(3.29 - 12.29)		1.730	5.64	(2.96 - 10.72)	
Abdominal lymph nodes on ultrasonography				<0.0001				<0.0001								
No		1				1										
Yes	1.345	3.84	(1.99 - 7.41)		1.309	3.70	(1.95 - 7.03)									
QFT result				0.0235								0.0249				
Negative		1														
Positive	1.279	3.60	(1.37 - 9.43)						1.2053	3.34	(1.36 - 8.21)					
Indeterminate	0.467	1.60	(0.81 - 3.16)						0.4099	1.51	(0.77 - 2.94)					

*intercept (constant)= -2.2894; † intercept=-2.0390; **intercept=-1.9091; ‡intercept=-1.6775

Figure S1. ROC curve for model 1 (A) and model 1 with Xpert (B) in model development dataset, and applying model 1 (C) and model 1 with Xpert (D) to the case control study

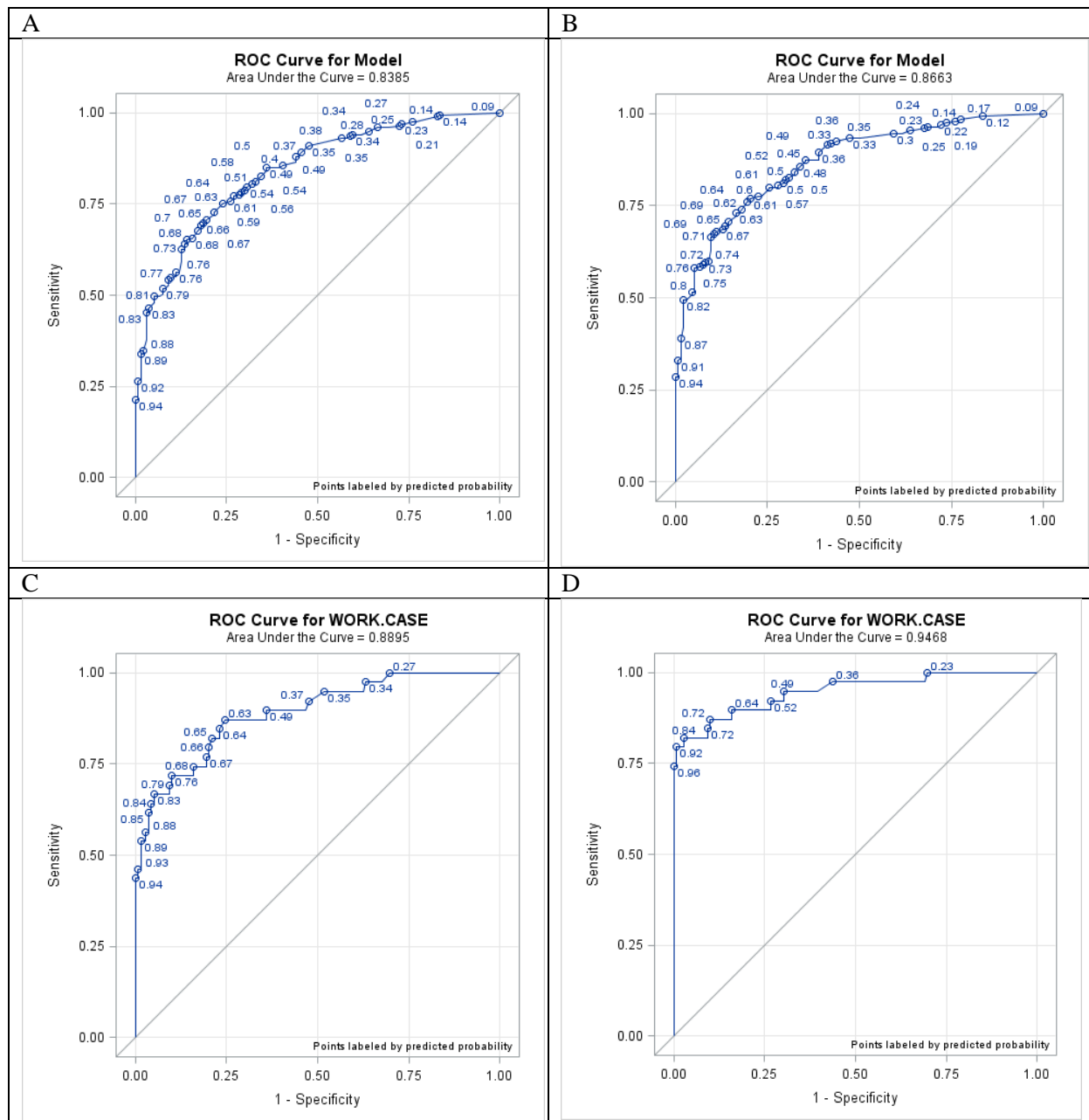
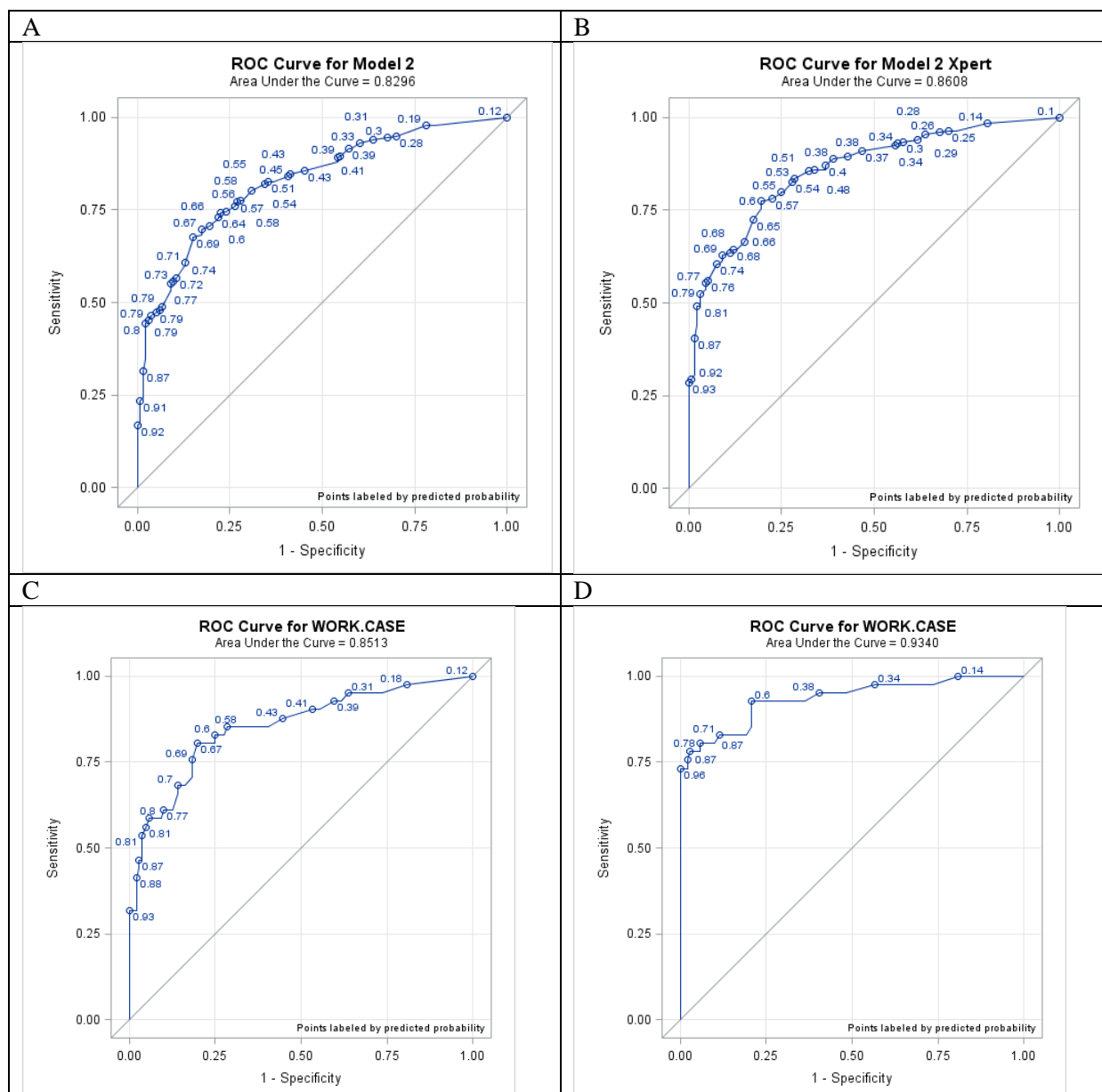


Figure S2. ROC curve for model 2 (A) and model 2 with Xpert (B) in model development dataset, and applying model 2 (C) and model 2 with Xpert (D) to the case control study



Appendix 5 – Clinical Case Definition for Classification of Intrathoracic Tuberculosis In HIV-Infected Children; details and definitions

Table 2. Details and Definitions for Research Evaluation and Reporting Purposes

	Definition guidelines	Definition for analysis in PAANTHER
Microbial confirmation	At least 1 positive culture (with confirmed Mycobacterium tuberculosis speciation) from sputum, which could be sampled from expectorated sputum, induced sputum, nasopharyngeal aspirates, gastric aspirates, or string tests (or other relevant intrathoracic samples).	Any positive culture (MGIT liquid culture and/or Lowenstein Jensen solid culture) identified as Mycobacterium tuberculosis on any type of sample, including stools samples or additional extra-pulmonary samples collected during the diagnostic procedures (before M0)
Clinical signs/symptoms suggestive of tuberculosis	Persistent cough (as defined below) Weight loss/failure to thrive (as defined below) Persistent unexplained fever (as defined below) Persistent, unexplained lethargy or reduced playfulness (as defined below) Additional signs and symptoms suggestive of TB in infants 0–60 days (or neonate) (as defined below)	Not defined on the basis of inclusion criteria but on signs and symptoms collected in anamnesis and during clinical examination
Persistent cough	Persistent (>2 weeks), non-remitting cough.	Cough lasting more than two weeks in anamnesis Report availability of data on cough evolution pattern
Weight loss/failure to thrive	unexplained weight loss: >5% reduction in weight compared with the highest weight recorded in last 3 months OR 1. Clear deviation from a previous growth trajectory and/or 2. Documented crossing of percentile lines in the preceding 3 months and/or 3. Weight for age z score of ≤ -2 in the absence of information on previous/recent growth trajectory and/or 4. Weight for height z score of ≤ -2 in the absence of information on previous/recent growth trajectory AND Not responding to nutritional rehabilitation (or antiretroviral therapy if HIV infected)	Weight for age z score of ≤ -2 OR Weight for height z score of ≤ -2 (calculated using the Stata module of WHO Anthro software; do not use reported z score)
Persistent unexplained fever	Persistent (>1 week) and unexplained fever (>38°C) reported by a guardian or objectively recorded at least once	Fever reported in anamnesis > 2 weeks

Persistent, unexplained lethargy or reduced playfulness	Persistent, unexplained lethargy or decrease in playfulness/activity reported by the parent/caregiver.	Fatigue reported in anamnesis > 2 weeks
Additional signs and symptoms suggestive of TB in infants 0–60 days (or neonate)	(a) Neonatal pneumonia or (b) Unexplained hepatosplenomegaly or (c) Sepsis-like illness	Consider only if a child aged < 60 days is enrolled. A clinical file review will be necessary. If other symptoms are present, it is not necessary to collect them.
CXR consistent with TB	Positive response for any 1 of the radiographic features, at the same location, by at least 2 expert reviewers. –CXR (2 views) will be read by a minimum of 2 independent and blinded readers who are experienced in reviewing CXRs in children. –The overall quality of the CXR will be indicated. –In the case of discordant reading, a third expert reader will be used and a final consensus achieved.	Interpretation of inclusion chest X-ray - Consider as primary experts Christophe Delacourt and National expert ; as secondary expert Laureline Berteloot - If no national expert, consider both French experts as primary experts - Discordance = discordant conclusion
History of exposure to M. tuberculosis	Reported exposure to a case of tuberculosis (household/close contact) within the preceding 24 months: –Documented (smear positive and/or culture positive, or tuberculosis treatment). or –Not documented but verbal report (smear positive and/or culture positive, or tuberculosis treatment).	Contact with anyone treated for TB last year or the year before in the anamnesis (if contact reported without period, consider as positive but report % of incomplete data)
immunological evidence of M. tuberculosis infection	–A positive tuberculin skin test (using 5TU PPD or 2TU RT23) defined as ≥10 mm if HIV uninfected. ≥5 mm if HIV infected or severely malnourished. or A positive IGRA test	Tuberculin skin test (CRF p. 10) with a transverse diameter ≥ 5 mm. Report delay between injection and results.
Response to anti-tuberculosis	1. Response to anti-tuberculosis therapy: clinical features suggestive of tuberculosis disease that were present at baseline have improved, and there is no new clinical feature suggestive of tuberculosis. or 2. No response to anti-tuberculosis therapy: clinical features	General condition improved at M2 visit AND/OR Respiratory symptoms and signs improved (Details of suggestive clinical features: improvement of cough, fever, fatigue, OR weight gain ≥ 10%)

	suggestive of tuberculosis disease that were present at baseline have not improved at 2 months of appropriate TB treatment.	
Appropriate anti-tuberculosis treatment	<p>Appropriate anti-tuberculosis treatment for presumed drug-susceptible tuberculosis should meet the following criteria:</p> <p>(a) Treatment with standard regimens in accordance with local or international tuberculosis treatment guidelines (define in protocol).</p> <p>(b) Satisfactory adherence proposed as 80% adherence by pill count or self-reported</p>	<p>TB regimen started consisting of a 3 or 4 drugs regimen including rifampicin (R), isoniazid (H), pyrazinamide (Z) +/- ethambutol (E) (RHEZ or RHZ)</p> <p>(consider real duration of treatment taken during the first 2 months)</p> <p>AND</p> <p>Adherence assessed as $\geq 80\%$ by pill count</p>

Titre : Amélioration du diagnostic de la tuberculose chez les enfants infectés par le VIH dans les pays à ressources limitées

Résumé : La tuberculose est une cause majeure de mortalité chez les enfants infectés par le VIH, représentant plus d'un tiers des décès chez ceux-ci. Il n'existe actuellement pas d'estimation précise de la prévalence du VIH dans le million de cas de tuberculose pédiatrique survenant chaque année. Cependant, les enfants co-infectés par le VIH pourraient représenter jusqu'à 1/5 de tous les décès dus à la tuberculose pédiatrique. Le diagnostic de tuberculose est difficile chez les enfants du fait de sa nature paucibacillaire et des difficultés de recueil d'échantillons respiratoires. Il constitue un obstacle majeur à l'accès au traitement. Plus de 96% des décès pourraient survenir chez des enfants non traités. Les enfants infectés par le VIH présentent des difficultés diagnostiques accrues et une mortalité plus élevée. Notre objectif principal était de contribuer à l'amélioration du diagnostic de la tuberculose chez les enfants infectés par le VIH par le développement d'un algorithme diagnostique. Ce travail est basé sur l'étude ANRS 12229 PAANTHER 01 qui a inclus 438 enfants séropositifs âgés de 0 à 13 ans suspects de tuberculose dans 8 hôpitaux du Burkina Faso, du Cambodge, du Cameroun et du Vietnam d'avril 2011 à mai 2014. La mortalité était élevée (19,6%) chez les enfants naïfs de traitement antirétroviral (TARV), malgré l'accès au traitement. Elle était plus élevée les cas confirmés par l'Xpert MTB/RIF (Xpert) ou la bacilloscopie. Le traitement antituberculeux était associé à une diminution de la mortalité chez les tuberculoses confirmées et non confirmées et à un retard problématique au début du TARV, celui-ci étant associé à une réduction massive de la mortalité quand débuté durant le premier mois de suivi. L'évaluation de la performance diagnostique de l'Xpert sur des échantillons bactériologiques alternatifs, y compris des aspirations naso-pharyngées (ANP), des échantillons de selles et le string test a montré que la combinaison d'un ANP et d'un échantillon de selles avait une faisabilité (100%) et une sensibilité (75,9%) élevées par rapport aux méthodes standards (crachats ou aspirations gastriques). L'algorithme diagnostique développé a inclus 11 prédicteurs dont des symptômes et des signes, le contage, les résultats de radiographie pulmonaire (RP) et d'échographie abdominale, ainsi que les résultats du Quantiferon Gold In-Tube (QFT). L'aire sous la courbe de ROC était de 0,866 lorsque l'Xpert était inclus. Le score développé à partir du modèle avait une bonne performance diagnostique globale. L'interprétation de la RP avait un accord inter-relecteurs médiocre à passable pour la détection de lésions compatibles avec la tuberculose chez les 403 enfants avec toutes les revues disponibles, et une précision diagnostique limitée (sensibilité 71,4%, spécificité 50,0%) pour le diagnostic de la tuberculose chez 51 cas et 151 contrôles vivants sans traitement à 6 mois. Enfin, nous présentons des données sur l'utilisation du standard de référence recommandé pour les études diagnostiques sur la tuberculose chez les enfants infectés par le VIH, décrivant les changements dans la classification résultant d'une mise à jour récente et soulignant les limites de ces standards. Ce travail souligne la nécessité d'un diagnostic rapide de la tuberculose chez les enfants infectés par le VIH afin de réduire la mortalité. Il propose d'améliorer le diagnostic par une approche alternative à la confirmation microbiologique et le développement d'un algorithme rapide de décision de traitement chez les enfants à haut risque de mortalité. Il met en évidence les défis pratiques et méthodologiques liés à l'utilisation de la RP et du standard de référence diagnostique dans cette population. Le projet TB Speed permettra la validation externe de l'algorithme chez les enfants infectés par le VIH et contribuera à l'amélioration du diagnostic de la tuberculose et à la réduction de la mortalité chez les enfants en décentralisant la confirmation microbiologique et le diagnostic dans des pays à faible revenu.

Mots-clefs : tuberculose, diagnostic, VIH, enfants, pays à ressources limitées, mortalité

Title: Improvement of tuberculosis diagnosis in HIV-infected children in low-income countries

Abstract: Tuberculosis remains a major cause of death in HIV-infected children, accounting for more than 1/3 of mortality in this population. There are currently no estimates of HIV prevalence in the one million childhood tuberculosis cases every year. However, it is estimated that HIV-related deaths account for up to 1/5 of all tuberculosis deaths in children. Due to its paucibacillary nature and difficulties in collecting respiratory specimen, tuberculosis diagnosis is challenging in children, and is a major barrier hampering access to treatment. It is estimated that more than 96% of tuberculosis death occur in children not receiving treatment. HIV-infected children present greater diagnostic challenges in a context of higher mortality. The main objective of this thesis was to contribute to the improvement of tuberculosis diagnosis in HIV-infected children by the development of a diagnostic algorithm. This thesis is based on the ANRS 12229 PAANTHER 01 study which enrolled 438 HIV-infected children aged 0 to 13 years presenting with a suspicion of tuberculosis in 8 hospitals from Burkina Faso, Cambodia, Cameroon, and Vietnam, from April 2011 to Mai 2014. Mortality was high [50 deaths (19.6%)] in the 266 art-naïve children from this study, despite access to antiretroviral and tuberculosis treatment. It was significantly higher in those with bacteriological confirmation by Xpert MTB/RIF (Xpert) or smear microscopy. Introduction of tuberculosis treatment led to decreased mortality in both confirmed and unconfirmed tuberculosis, and to delayed ART introduction, which is a problem, as ART was associated with a more than 10-fold reduction in mortality when started during the first month of follow-up. The assessment of the diagnostic performance of Xpert on alternative specimens including nasopharyngeal aspirates (NPA), stool samples, and string tests showed that a combination of NPA and stool samples had a high feasibility (100%) and sensitivity (75.9%) and a yield similar to standard specimen collection methods consisting in sputum samples or gastric aspirates. We developed a tuberculosis diagnostic algorithm for HIV-infected children. The model identified 11 predictors including some symptoms and signs, history of contact, chest radiograph (CXR) and abdominal ultrasonography findings, and Quantiferon TB Gold In-Tube (QFT) results. Its area under the receiver operating characteristic curves was 0.839, and 0.866 when Xpert was included. The score developed from the model had a good diagnostic performance overall. CXR interpretation showed poor to fair inter-reader agreement for the detection of lesions consistent with tuberculosis in 403 children with all reviews available, and limited diagnostic accuracy (sensitivity 71.4%; specificity 50.0%) for the diagnosis of tuberculosis in 51 culture confirmed cases and 151 controls alive without treatment at 6 months. At last, we present data on the use of the recommended tuberculosis reference standards for diagnostic studies in HIV-infected children, describing changes in classification occurring from a recent update and highlighting limitations of these reference standards. Overall, this work contributes to highlight the need for an accurate and timely tuberculosis diagnosis in HIV-infected children to reduce mortality. It proposes two major ways to improve diagnosis by an alternative approach to microbiological confirmation and by an algorithm for prompt treatment decision in children with a high risk of mortality. It also highlights practical and methodological challenges related to the use of CXR and diagnostic reference standards in this population. The newly funded TB Speed project will enable external validation of the algorithm in HIV-infected children. By decentralizing Xpert performed on NPA and stool in district health systems in low-income countries, it will further contribute to the overall goal of improving tuberculosis diagnosis and reducing mortality in children.

Key words: tuberculosis, diagnosis, HIV, children, mortality, low income countries, mortality

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