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ABSTRACT

Objective: To evaluate the feasibility of the implementation of a commercial rapid molecular diagnostic test (Xpert MTB/RIF) for the routine diagnosis of smear-negative or extrapulmonary tuberculosis (TB) and its diagnostic accuracy, and to assess HIV prevalence in a real-life setting in Madagascar. This study was set in a tertiary care hospital in Madagascar.
Methods: A prospective cohort study was conducted of all consecutive cases with suspected smear-negative and/or extrapulmonary TB over a 2-year period. Cases were classified as proven, probable, or possible TB cases, or as having an alternative diagnosis.
Results: Of the 363 patients included, 183 (50.4%) had suspected smear-negative pulmonary TB and 180 (49.6%) had suspected extrapulmonary TB. For proven cases, the sensitivity, specificity, positive and negative predictive values of Xpert MTB/RIF were 82.4%, 98.8%, 98.3%, and 86.6%, respectively; for proven and probable cases grouped together, these values were 65%, 98.8%, 98.5%, and 64%, respectively. The diagnostic accuracy was slightly lower for extrapulmonary TB compared to smear-negative pulmonary TB. The prevalence of HIV infection was 12.1%, but almost half of these cases did not have TB (alternative diagnosis group).
Conclusions: The implementation of a rapid diagnosis programme for TB in a resource-poor setting is feasible. The performance of the Xpert-MTB/RIF was remarkable in this difficult-to-diagnose population. HIV prevalence in this study was much higher than the prevalence reported in the general population in Madagascar, in patients with TB and patients with conditions other than TB.

1. Introduction

Tuberculosis (TB) is a major public health threat in Madagascar (Rakotonirina et al., 2016), with an estimated prevalence of 442 per 100 000 inhabitants (95% confidence interval (CI) 22–735) and an estimated incidence of 234 per 100 000 inhabitants (95% CI 193–280), which are among the highest rates worldwide (World Health Organization, 2014a). Approximately one-third of all TB cases are extrapulmonary and another third are smear-negative. Antananarivo is the most populous city in Madagascar

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and TB is also a major health concern here (Rakotosamimanana et al., 2014).

The diagnosis of smear-negative and extrapulmonary TB remains challenging in resource-limited settings. In Madagascar, there are also difficulties related to the start of anti-TB therapy, which is given as a priority to cases with proven pulmonary TB (smear-positive sputum) due to epidemiological issues in order to decrease transmission. However, the confirmation of smear-negative and extrapulmonary TB cases requires a positive *Mycobacterium tuberculosis* culture, which may take up to 8 weeks. Patients with the most serious clinical presentations of extrapulmonary TB (such as those with central nervous system or digestive system involvement) are frequently too ill to wait for diagnostic confirmation. In this context, rapid tests for the diagnosis of TB have an impact on clinical outcomes. Although recent data are lacking, multidrug resistance is considered low in Madagascar and is reported to be approximately 0.5% for patients with a new diagnosis of TB and 3% for previously treated patients (Ramarokoto et al., 2010).

In contrast to other African countries, the prevalence of HIV infection is estimated to be less than 1% in Madagascar (Frickmann et al., 2013; Leutscher et al., 2005), representing approximately 50 000 to 60 000 HIV-infected individuals. However, by the end of 2015, only around 2000 people were diagnosed and less than 1000 were receiving antiretroviral therapy. A significant proportion of new diagnoses are late presenters with opportunistic infections at the time of HIV diagnosis, such as cryptococcal meningitis or disseminated TB.

To improve and accelerate the diagnosis of smear-negative and extrapulmonary TB, molecular-based techniques, such as Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA), are useful tools. Xpert MTB/RIF has been validated in a series of small studies for both smear-negative and extrapulmonary TB, showing a wide range of sensitivities and specificities according to the sample evaluated, but with an overall good performance (Maynard-Smith et al., 2014; Piatek et al., 2013). However, studies validating Xpert MTB/RIF according to strict clinical case definitions and in a real-life setting are scarce.

The aim of this study was to evaluate the operational feasibility of the implementation of a routine molecular rapid test (Xpert MTB/RIF) for the diagnosis of TB and its performance for suspected cases of smear-negative and extrapulmonary TB in a real-life setting in Madagascar. It was also aimed to estimate HIV prevalence in this at-risk population.

2. Methods

The Joseph Raseta Befelatanana Hospital (HUJRB) is a 350-bed tertiary referral university hospital located in Antananarivo, the capital city of Madagascar. It includes all medical specialties and an intensive care medicine department.

2.1. Patient population

All patients with suspected smear-negative and/or extrapulmonary TB (adult patients with a febrile illness and chronic respiratory symptoms, pleural effusion, chronic abdominal pain or ascites, chronic meningitis, or other symptoms suggestive of extrapulmonary TB) presenting or referred to the infectious diseases, pulmonology, gastroenterology, or neurology services of HUJRB were eligible for inclusion in the study. Patients who contributed both pulmonary and extrapulmonary samples were considered as extrapulmonary cases.

All suspected TB cases at inclusion were re-evaluated at the end of the study by an international panel of three infectious diseases specialists (validation team) not directly involved in the inclusion process or the follow-up of cases. Following this evaluation, patients were classified into the following four clinical categories: (Rakotonirina et al., 2016) proven TB: culture-positive for *M. tuberculosis* in any sample; (World Health Organization, 2014a) probable TB: culture-negative, but with clinical data compatible with TB and a clinical response to anti-TB specific treatment in the absence of other antimicrobial therapy; (Rakotosamimanana et al., 2014) possible TB: culture-negative, but clinical and biological data compatible with TB; clinical evolution on anti-TB therapy not available, but no other alternative diagnosis; (Ramarokoto et al., 2010) alternative diagnosis: another clinical condition was confirmed and retained and there was no later evidence suggesting TB.

The criteria of Marais et al. were used for the diagnosis of tuberculous meningitis (Marais et al., 2010).

This classification and stratification of cases was independent of Xpert MTB/RIF and the panel was blinded to these results.

2.2. Diagnostic tests

For suspected pulmonary TB patients, direct microscopic examination of sputum smears after Ziehl–Neelsen staining was performed at HUJRB. All negative sputum acid-fast bacillus (AFB) smears from pulmonary TB patients and specimens from extrapulmonary TB patients were sent to the Mycobacteria Laboratory at the Institut Pasteur de Madagascar for a second smear examination, culture, and Xpert MTB/RIF screening.

For all biological fluids (sputum, gastric aspirate, urine, cerebrospinal fluid, pleural effusion, peritoneal fluid), 0.5 ml was used for the Xpert MTB/RIF test, which was performed according to the manufacturer's instructions. The remaining samples were decontaminated by sodium lauryl sulphate method (Tacquet and Tison, 1961). Biopsies were ground and diluted in distilled water, and 0.5 ml was used for the Xpert MTB/RIF assay. The remaining samples were decontaminated with H_2SO_4 (David et al., 1989). Decontaminated specimens were examined under a fluorescence microscope and the remaining sample cultured on standard Löwenstein–Jensen medium. Mycobacterial isolates were identified according to growth on the medium, colony morphology, and the SD Bioline TB Ag MPT64 test. Drug susceptibility tests were performed using the indirect proportion method (Canetti et al., 1963).

Standard analyses (cytological and biochemical) for biological fluids (cerebrospinal fluid, peritoneal fluid, pleural effusion) and the pathological examination of tissue samples (lymph nodes, cutaneous biopsies, pleural and synovial biopsies) were also performed at HUJRB. HIV serology testing was offered to all patients. HIV status was confirmed using three different conformational rapid tests, according to national recommendations. Xpert MTB/RIF and all other standard microbiological and pathological test results were available in real-time and used to assist the clinicians in making medical decisions.

2.3. Data management and statistical analyses

A specific case report form was created for the study and all data were collected in a dedicated database. The Chi-square test and Fisher's exact test were used as appropriate to compare categorical variables. Continuous variables were compared between subgroups using the Mann–Whitney test. All p-values were considered significant at $p < 0.05$. The statistical analyses were performed using IBM SPSS and R software. The performance of the diagnostic methods was tested using two gold standards: (Rakotonirina et al., 2016) proven cases, and (World Health Organization, 2014a) proven and probable cases grouped together. Exact binomial confidence limits were calculated for test sensitivity, specificity, and positive and negative predictive values (PPV and NPV). Youden's index was calculated for Xpert MTB/RIF.

Table 1
Baseline characteristics of the study patients and final classification of suspected TB cases

	All cases $n = 363$ (%)	Suspected pulmonary TB cases $n = 183$ (%)	Suspected extrapulmonary TB cases[a] $n = 180$ (%)	p-Value
Sex ($n = 363$)				0.099
Female	131 (36.1%)	58 (31.7%)	73 (40.6%)	
Male	232 (63.9%)	125 (68.3%)	107 (59.4%)	
Age ($n = 363$), median (SD) years	38.7 (15.2)	40.0 (15.3)	37.3 (15.0)	0.082
HIV serological status ($n = 314$)				0.206
Negative	276 (87.9%)	140 (85.4%)	136 (90.7%)	
Positive	38 (12.1%)	24 (14.6%)	14 (9.33%)	
Previously known as HIV-positive	16 (42.1%)	11 (45.8%)	5 (35.7%)	
Classification of suspected cases ($n = 363$)				<0.001
Confirmed TB	119 (32.8%)	69 (37.7%)	50 (27.8%)	
Probable TB	41 (11.3%)	10 (5.46%)	31 (17.2%)	
Possible TB	56 (15.4%)	20 (10.9%)	36 (20.0%)	
Alternative diagnosis	147 (40.5%)	84 (45.9%)	63 (35.0%)	

TB, tuberculosis; SD, standard deviation.
[a] Including disseminated disease (pulmonary and extrapulmonary clinical presentation).

Table 2
Overall results for direct examination, culture, and Xpert MTB/RIF for the 438 samples from 363 patients with suspected smear-negative pulmonary or extrapulmonary TB

	All samples $n = 438$ (%)	Pulmonary samples $n = 205$ (%)	Extrapulmonary samples $n = 233$ (%)	p-Value
Direct examination[a] ($n = 438$)				<0.001
Negative	397 (90.6%)	173 (84.4%)	224 (96.1%)	
Positive	41 (9.36%)	32 (15.6%)	9 (3.86%)	
Culture[b] ($n = 438$)				<0.001
Negative	299 (68.3%)	122 (59.5%)	177 (76.0%)	
Positive	139 (31.7%)	83 (40.5%)	56 (24.0%)	
Xpert MTB/RIF ($n = 437$)				<0.001
Negative	298 (68.2%)	121 (59.3%)	177 (76.0%)	
Positive	139 (31.8%)	83 (40.7%)	56 (24.0%)	

TB, tuberculosis.
[a] Ziehl-Neelsen stain
[b] Löwenstein–Jensen medium.

2.4. Ethics

The study was approved by the National Ethics Committee of the Ministry of Health of Madagascar (No. 049-MSANP/CE). All patients gave informed consent before any procedure was performed.

3. Results

3.1. Patients

Between September 2013 and June 2015, 363 patients were included and a total of 438 samples were analysed. Two hundred and ninety-eight (82.1%) patients contributed one sample, 56 (15.4%) contributed two, and nine (2.38%) contributed three or more samples. Among the 56 patients with two studied samples, six (10.7%) had two pulmonary specimens, 33 (58.9%) had one pulmonary and one extrapulmonary specimen, and 17 (30.4%) had two extrapulmonary specimens. All cases with three or more specimens had at least one pulmonary and one extrapulmonary specimen.

The baseline characteristics of the study patients and the final classification of suspected cases are shown in Table 1. In brief, 183 (50.4%) patients had suspected pulmonary TB and 180 (49.6%) had suspected extrapulmonary TB or mixed pulmonary/extrapulmonary TB. Overall, proven cases accounted for 119 (32.8%) patients, probable cases for 41 (11.3%), and possible cases for 56 (15.4%). An alternative diagnosis was identified in 147 cases (40.5%). The median age of the patients was 38.7 years (standard deviation 12.1 years) and 232 (63.9%) were male. The distribution of proven, probable, possible, and alternative diagnoses differed according to the clinical presentation (pulmonary vs. extrapulmonary, $p < 0.001$).

In 38 of 314 cases, the HIV serology was positive, giving an overall prevalence of 12.1%. Sixteen cases (45.7%) were known to be HIV-positive at study entry and 22 (54.3%) were diagnosed in the setting of the study. Thirteen cases (34.2%) had proven TB, five cases (13.2%) had probable TB, and four cases (10.5%) had possible TB. Sixteen of 38 HIV cases (42.1%) were diagnosed among patients for whom an alternative diagnosis was available. The CD4 cell count was available for 34 cases (89.5%), with a median (interquartile range) value of 108 (38–212) cells/mm^3. HIV serology could not be performed in 32 (8.8%) of 363 patients, and it was refused by the patient in 17 cases (4.7%).

3.2. Samples

Of the 438 samples included, 205 (46.8%) were respiratory samples (sputum and/or gastric aspirates) and 233 (53.2%) were extrapulmonary samples (Table 2). A negative Ziehl–Neelsen sputum stain was a condition at screening for study inclusion. An auramine stain was then performed on the new research sample and tested positive in 32 (15.6%) respiratory specimens. Nine (3.82%) extrapulmonary samples were also auramine-positive.

Suspected extrapulmonary TB cases provided a large variety of clinical specimens. The most frequent were cerebrospinal fluid in 77 (33%) cases, ascitic fluid in 62 (26.6%), and pleural effusion in 50 (21.5%) cases. Among the remaining 44 samples (18.9%), the most frequent were lymph nodes ($n = 16$), urine ($n = 8$), subcutaneous abscesses ($n = 6$), and pleural biopsies ($n = 6$). Culture and Xpert MTB/RIF had an overall positivity rate of around 40% for pulmonary TB and 24% for extrapulmonary TB (Table 2).

For the 56 (15.4%) cases with more than one sample studied, 28 (50%) cases had two negative samples, 15 cases (26.8%) had one

Table 3
Performance of Xpert MTB/RIF for the 438 samples from 363 patients with suspected smear-negative pulmonary or extrapulmonary TB; value and 95% confidence interval for all estimations

	Gold standard A[a]	Gold standard B[b]
All samples (n = 438)		
SE	0.824 (0.751; 0.883)	0.652 (0.582; 0.717)
SP	0.988 (0.956; 0.999)	0.988 (0.956; 0.999)
PPV	0.983 (0.941; 0.998)	0.985 (0.948; 0.998)
NPV	0.866 (0.808; 0.911)	0.694 (0.63; 0.753)
Youden's index	0.812 (0.708; 0.881)	0.64 (0.539; 0.716)
Smear-negative pulmonary samples (n = 205)		
SE	0.867 (0.775; 0.932)	0.832 (0.741; 0.901)
SP	0.989 (0.938; 1)	0.989 (0.938; 1)
PPV	0.986 (0.926; 1)	0.988 (0.932; 1)
NPV	0.888 (0.808; 0.943)	0.845 (0.76; 0.909)
Youden's index	0.856 (0.71; 0.932)	0.82 (0.679; 0.9)
Extrapulmonary samples (n = 233)		
SE	0.763 (0.634; 0.864)	0.495 (0.398; 0.593)
SP	0.987 (0.928; 1)	0.987 (0.928; 1)
PPV	0.978 (0.885; 0.999)	0.982 (0.903; 1)
NPV	0.841 (0.748; 0.91)	0.574 (0.484; 0.66)
Youden's index	0.749 (0.562; 0.863)	0.482 (0.326; 0.592)

TB, tuberculosis; SE, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value.
[a] Proven cases (culture-positive cases).
[b] Proven and probable cases (culture-positive cases or clinical response to anti-TB treatment without any other diagnosis or other treatment).

positive and one negative result, and 13 cases (23.2%) had two positive samples. In 57% of cases with a pulmonary and an extrapulmonary sample studied, at least one was positive, compared to 41% of cases with two extrapulmonary samples and 33% of cases with two pulmonary samples analysed (p = 0.310). For the nine cases (2.38%) with three or more samples studied, all samples were negative in six cases (66.7%) and one or two samples were positive in three cases (33.3%).

3.3. Performance of diagnostic tests

The sensitivity, specificity, PPV, and NPV of Xpert MTB/RIF are shown in Table 3. Xpert MTB/RIF was analysed using the microbiology gold standard (proven cases, positive culture), as well as the alternate gold standard (proven/probable cases, positive culture or clinical response without an alternative diagnosis or combined treatments). Overall, Xpert MTB/RIF showed a sensitivity of 0.824 (95% CI 0.751; 0.883) and an extremely high specificity of 0.988 (95% CI 0.956; 0.999). The sensitivity was lower for extrapulmonary TB than for pulmonary TB. For extrapulmonary samples, analyses were performed for the three most prevalent types (cerebrospinal fluid, ascitic fluid, and pleural effusion) and other samples (Table 4). The overall rate of positive and negative results according to the clinical category (proven, probable, possible, or alternative diagnosis) is shown in Figure 1.

Table 4
Performance of Xpert MTB/RIF in 233 extrapulmonary samples from patients with suspected extrapulmonary TB; value and 95% confidence interval for all estimations

	Gold standard A[a]	Gold standard B[b]
Cerebrospinal fluid samples (n = 77)		
SE	0.692 (0.386; 0.909)	0.444 (0.255; 0.647)
SP	0.933 (0.817; 0.986)	1 (0.838; 1)
PPV	0.75 (0.428; 0.945)	1 (0.64; 1)
NPV	0.913 (0.792; 0.976)	0.674 (0.52; 0.805)
Youden's index	0.626 (0.203; 0.895)	0.444 (0.093; 0.647)
Ascitic fluid samples (n = 62)		
SE	0.643 (0.351; 0.872)	0.407 (0.224; 0.612)
SP	0.929 (0.765; 0.991)	1 (0.698; 1)
PPV	0.818 (0.482; 0.977)	1 (0.615; 1)
NPV	0.839 (0.663; 0.945)	0.484 (0.302; 0.669)
Youden's index	0.571 (0.116; 0.864)	0.407 (-0.078; 0.612)
Pleural effusion samples (n = 50)		
SE	0.636 (0.308; 0.891)	0.241 (0.103; 0.435)
SP	1 (0.842; 1)	1 (0.681; 1)
PPV	1 (0.473; 1)	1 (0.473; 1)
NPV	0.889 (0.739; 0.969)	0.389 (0.231; 0.565)
Youden's index	0.636 (0.15; 0.891)	0.241 (-0.217; 0.435)
Other samples (n = 44)[c]		
SE	0.952 (0.762; 0.999)	0.923 (0.749; 0.991)
SP	0.75 (0.509; 0.913)	0.933 (0.681; 0.998)
PPV	0.8 (0.593; 0.932)	0.96 (0.796; 0.999)
NPV	0.938 (0.698; 0.998)	0.875 (0.617; 0.984)
Youden's index	0.702 (0.27; 0.912)	0.856 (0.429; 0.989)

TB, tuberculosis; SE, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value.
[a] Proven cases (culture-positive cases).
[b] Proven and probable cases (culture-positive cases or clinical response to anti-TB treatment without any other diagnosis or other treatment).
[c] Including lymph nodes, urine, subcutaneous abscesses, pleural biopsies.

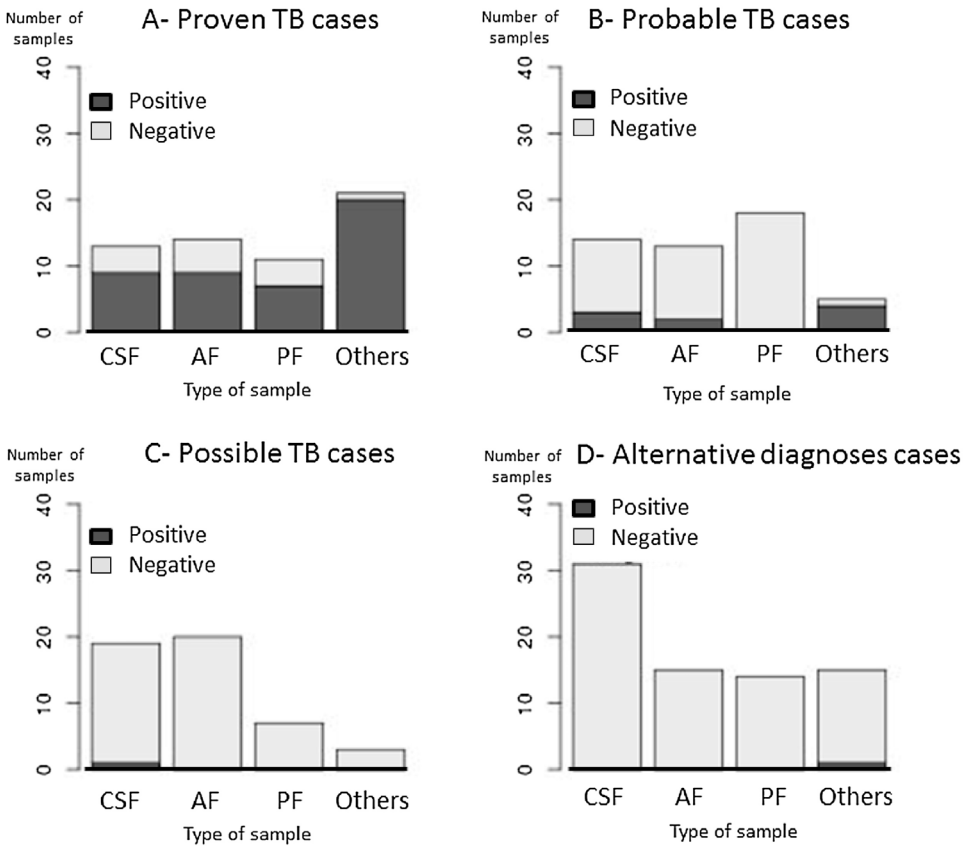

Figure 1. Positivity rate of Xpert MTB/RIF according to the classification of cases (proven, probable, or possible TB and alternative diagnoses) and type of extrapulmonary specimen.
CSF: cerebrospinal fluid; AF: ascitic fluid; PF: pleural fluid; others: other sample types (including lymph nodes, urine, subcutaneous abscesses, pleural biopsies, synovial fluid, pericardial fluid, and bone marrow aspirates.).

3.4. M. tuberculosis *resistance to anti-TB drugs*

Resistance to rifampicin was detected by Xpert MTB/RIF in two of 363 cases (0.55%): a 62-year-old woman with peritoneal TB and a positive Xpert MTB/RIF in ascitic fluid, and a 28-year-old man with smear-negative pulmonary TB. In addition, nine cases were reported as undetermined (five were culture-negative and four culture-positive with no resistance detected by conventional methods). All other specimens were negative for rifampicin resistance.

4. Discussion

This study conducted in Madagascar (the MadaXpert study) confirmed the public health problem and burden of smear-negative and extrapulmonary TB. In this resource-poor setting, the implementation of a rapid molecular diagnostic test for TB in a real-life situation was feasible and provided a reliable and rapid diagnosis of TB. Overall, Xpert MTB/RIF showed a high sensitivity and an extremely high specificity, thus confirming its excellent performance for the diagnosis of both smear-negative pulmonary TB and extrapulmonary TB in a wide range of specimens when compared to culture. These results were obtained in a public reference hospital in the context of acutely ill patients presenting with a clinical suspicion of TB. An HIV seroprevalence of 12% was identified in this high-risk population, which is much higher than the overall reported prevalence of around 1% for the general population. Similar to previous reports, drug-resistant *M. tuberculosis* was very infrequent (Ramarokoto et al., 2010).

Sputum smear microscopy remains a common method to diagnose pulmonary TB cases, but lacks sensitivity. Moreover, it does not allow for the diagnosis of extrapulmonary cases, which remains a challenging issue for clinicians (Steingart et al., 2007). Indeed, extrapulmonary TB is frequently underestimated due to diagnostic difficulties (Kulchavenya, 2014), although it represents a significant proportion of TB cases in some countries (Gunal et al., 2011). Nucleic acid amplification tests have facilitated the diagnosis of some extrapulmonary TB forms, but sensitivity is poor for some of them (Ketata et al., 2015; Mehta et al., 2012). Although Xpert MTB/RIF has been largely validated for the diagnosis of pulmonary TB (Steingart et al., 2014), extrapulmonary clinical specimens still need more attention (Maynard-Smith et al., 2014).

In the present study, Xpert MTB/RIF showed a sensitivity of 76.3% for extrapulmonary TB. It was able to rapidly identify around 65–70% of confirmed meningeal, abdominal, and pleural TB cases; this represents a huge clinical impact in terms of providing timely life-saving treatment. The overall results for Xpert MTB/RIF were quite similar to those reported in the literature (Bates and Zumla, 2016). A proportion of gastric aspirates were included among the respiratory samples. This sample type has been validated in children (Bates et al., 2013), but has been poorly studied in adults. In the present study, when more than one sample per patient was included, there was a trend towards a higher positivity rate in cases with both pulmonary and extrapulmonary specimens analysed, although this was not statistically significant.

In contrast to neighbouring continental African countries, such as Mozambique, the prevalence of HIV in Madagascar is believed to be less than 1% in the general population (Frickmann et al., 2013; Leutscher et al., 2005). However, recent large studies are lacking and the possibility that a significant proportion of cases are

undiagnosed cannot be excluded. Although some cases included in this study were already known to be HIV-positive, most cases were diagnosed during the study period. The high HIV prevalence in patients with suspected TB in this study is not totally unexpected. However, 42.1% of HIV diagnoses were performed in patients classified by the expert validation team as having an alternative diagnosis. These data suggest that HIV seroprevalence in Madagascar may be underestimated and call for large HIV testing programmes to be put in place. Most HIV cases presented with advanced disease and a median CD4 cell count of around 100 cells/mm^3. HIV serology was part of the initial evaluation of the cases in the study, but in almost 10% of cases it could not be performed due to a lack of tests during some of the time periods. In addition, approximately 5% of patients refused to be tested.

The physicians' perception of the utility of this rapid diagnostic tool was extremely positive; it was concluded that Xpert MTB/RIF represented a major addition in assisting clinical decision-making and case management. The sensitivity of Xpert MTB/RIF for extrapulmonary TB was 76.3% in this study and was close to 59% for both confirmed and probable cases when grouped together. In Madagascar, where TB is a major public health issue, anti-TB treatment is prioritized for pulmonary cases in order to decrease transmission. Thus, unconfirmed extrapulmonary cases have sometimes remained untreated for long periods of time. Some extrapulmonary forms of TB, such as tuberculous meningitis, are among the most serious, and a delay in the initiation of treatment is associated with a poor prognosis in terms of mortality or serious sequelae in survivors. In these cases, the implementation of programmes allowing a rapid diagnosis has major clinical implications (Piatek et al., 2013).

This study has several strengths. First, most studies evaluating the performance of a microbiology test have included limited clinical information. In this study, each case was thoroughly evaluated to include not only those with microbiologically confirmed TB, but also all cases that most probably had TB but remained unconfirmed. Moreover, the strict clinical follow-up allowed the exclusion of all cases with a documented alternative diagnosis, thus providing excellent negative controls for the evaluation of the performance of the tests. Second, with almost 400 patients included over 2 years, the sample size provides a representative overview of the TB perspective in Antananarivo and overall in Madagascar, as well as sufficient power to evaluate the diagnostic techniques. Finally, this study provided data on HIV prevalence in a high-risk population in Madagascar. As a limitation, the heterogeneous types of extrapulmonary sample precluded the analysis of specific clinical presentations due to the small numbers of cases for the different TB types. In addition, it was not possible to detect the impact of the early diagnosis of these severe forms of TB on mortality, as empirical treatment was available.

In conclusion, this study – the MadaXpert study – confirmed the feasibility and usefulness of the implementation of a routine rapid molecular diagnosis programme for TB in Madagascar. Xpert MTB/RIF demonstrated a good performance for all forms of difficult-to-diagnose TB, comparable to reports from previous studies. HIV prevalence was high in this high-risk population. *M. tuberculosis* resistance to anti-TB drugs was very low. A scale-up of the Xpert MTB/RIF rapid TB diagnostic method should be encouraged in low-income countries with a high TB prevalence (World Health Organization, 2014b) given its ability to overcome operational concerns.

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