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Contribution of Gene-Xpert in the Diagnosis of Negative Microscopy Pulmonary Tuberculosis in Brazzaville, Republic of Congo

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ABSTRACT

Setting: Xpert MTB/Rif has become an integral part of tuberculosis diagnostic algorithms in many low- and middle-income coun-tries and no published data was retrieved regarding the diagnostic accuracy of Xpert MTB/RIF in sputum smear-negative pulmonary TB in RoC.

Objectives: This study aimed at determining the diagnostic accuracy of Xpert MTB/RIF in diagnosing sputum smear-negative pulmo-nary tuberculosis keeping AFB microscopy as the gold standard.

Design: For 4 months, one morning sputum smear was performed for every TB suspect (cough lasting >3 weeks, as defined in Congolese's national guidelines) with AFB microscopy and GeneXpert.

Results: Of 90 TB suspects for whom AFB microscopy and GeneXpert were performed, 79 (87.78%) sputum smear-negative 17 (21.52%) GeneXpert positive cases were identified. During the study, 11 (12.22%) sputum smear-positive cases were diag-nosed. GeneXpert has greatly contributed to increasing the total number of bacteriologically proven cases.

Conclusion: These results confirms the utility of GeneXpert in the diagnosis of tuberculosis especially smear negative pulmonary tuber-culosis, and demonstrates that the expected number of smear-negative TB cases in Republic of Congo is probably much higher than the World Health Organization's current annual estimates.

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Introduction

Republlic of Congo, with these 5.2 million inhabitants, has low access to pulmonary tuberculosis diagnosis, with a rate of mortality of 57 (32–89) per 100,000 inhabitants due to TB occur [1]. Sputum smear examination is the mainstay of pulmonary TB diagnosis [2]. In a few studies performed in Brazzaville, there is a higher proportion of bacteriological-ly-confirmed TB, a shorter time to treatment initiation [3,4]. For several years, the diagnosis of pulmonary tuberculosis is done through clinical signs, results from sputum smear mi-croscopy, tuberculin skin test and X-ray chest [2].

According to the National Tuberculosis Control Program, 11,512 cases of all forms of tuberculosis were detected in 2012, of which 4,465 cases of pulmonary tuberculosis with smear sputum positive microscopy, 39%, 3,937 cases of pul-monary tuberculosis with smear sputum negative microsco-py [5]. A positive smear signifies a very large bacterial pop-ulation in the lung lesions whereas several negative smears suggest a smaller bacterial load [6,7]. Such smear-negative cases do not require the same intensity and duration of treatment as smear-positive cases. Diagnostic difficulties arise when sputum smears are negative for acid-fast bacilli in tuberculin-positive patients with compatible symptoms and chest radiographs for tuberculosis. Microscopy requires approximately 5,000 to 10,000 AFB/ml of sputum for detec-tion [8]. Many of these smear-negative patients yield positive cultures for M tuberculosis, whereas others remain culturally negative.

The eligibility criteria for the GeneXpert test are set up such that only the cases of the patients in therapeutic failure are diagnosed with this new molecular tool, because of reagents in reduced number [9]. Studies have consistently shown that Xpert MTB/RIF can identify a substantial proportion of smear-negative TB patients and extrapulmonary TB, partic-ularly in people co-infected with human immunodeficiency virus [10-12].

Drug-resistant TB care, the number of MDR/RR-TB cases among notified pulmonary TB cases estimated 290. Globally, there were an estimated 2.4% of new cases and 12% of previ-ously treated cases with MDR/RR-TB. All of cases previously treated were tested for rifampicin resistance and only 15% of cases were

primary resistances [1].

To improve patient care and decrease TB transmission, ac-curate and quick detection of TB especially smear negative and TB drug resistance is essential [13]. Xpert can serve as a time-saving diagnostic method for microbiological diagno-sis of smear-negative TB in countries with a low TB preva-lence [14].

In national TB programs, low bacillary load pulmonary tuberculosis is of little interest because of its low risk of contamination. However, clinically, it is a concern due to the prognostic importance of anti-tuberculosis treatment. Improving the diagnostic accuracy and reducing diagnostic delay in both smear-negative pulmonary tuberculosis is therefore paramount [14,15]. Also, the diagnosis of smear-negative pulmonary tuberculosis still represents a challenge, due to its paucibacillary nature leading to a prolonged time before the diagnosis occur [14]. Xpert MTB/Rif has become an integral part of tuberculosis diagnostic algorithms in many low- and middle-income countries and no published data was re-trieved regarding the diagnostic accuracy of Xpert MTB/RIF in sputum smear-negative pulmonary TB in RoC. This study thus aimed at determining the diagnostic accuracy of Xpert MTB/RIF in diagnosing sputum smear-negative pulmonary tuberculosis keeping AFB microscopy as the gold standard.

Material and Methods

A descriptive, analytical study was conducted from July to November 2019, during 4 months, at the military hospital, in Brazzaville. This research study conducted on 79 sputum specimens from bacteriologically confirmed cases sputum smear negative. These patients came from Brazzaville. These patients were initially screened for TB through AFB sputum smear microscopy at medical laboratory of the military hos-pital. All the AFB smear-negative patients with persistent clinical symptoms of TB were referred to the confirmation of test GeneXpert MTB/RIF assay.

Before specimen collection, the patients were informed about the study and written consent was obtained. The pa-tients were asked to collect the sputum specimens following the standard sputum collection technique using the recom-mended sterile plastic (50-mL Tarson) containers. One half of the collected sputum specimen was used for AFB sputum smear microscopy, while the remaining portion was used for GeneXpert MTB/RIF assay. The research study was conduct-ed following the approved guidelines framed by the research scrutiny committee of the Institut National de Recherche en Science de la Santé (IRSSA).

Ziehl-Neelsen staining and the Xpert test was performed according to the manufacturer's recommendations. With phased introduction of Xpert to replace sputum smears as the initial diagnostic test for new pulmonary TB [16]. In the smear microscopy, up to two sputum smears per patient were examined by conventional optic microscopy based on direct Ziehl-Neelsen staining, as per routine [17].

Direct smears were prepared for microscopic examination of AFB by applying one drop of sputum samples on a clean glass slide which was labeled with the ID number. The smear was air dried followed by heat fixation and staining using ZN staining methods [18]. The stained smears were examined microscopically using 100× objective lens. Control slides (known positive and known negative) were included as pro-cedural control with each run of ZN staining.

In the Xpert, usually only one of the submitted samples was examined within two days of being received by the laborato-ry. To 2 mL of sample reagent, 1 mL of sputum specimen was added and the sputum specimen was allowed to liquefy for 15 min. using a sterile dropper provided with the kit, 2 mL of mixture was transferred into the GeneXpert cartridge. This cartridge contained the required reagents for nucleic acid amplification and RIF drug resistance detection. The inocu-lated cartridge was loaded into the GeneXpert machine. Re-sults were displayed by the GeneXpert system automatically within 2 h. The operator can read and print the test results as "MTB detected; RIF resistance not detected." Confirmed bacteriological TB was defined as a positive Xpert result.

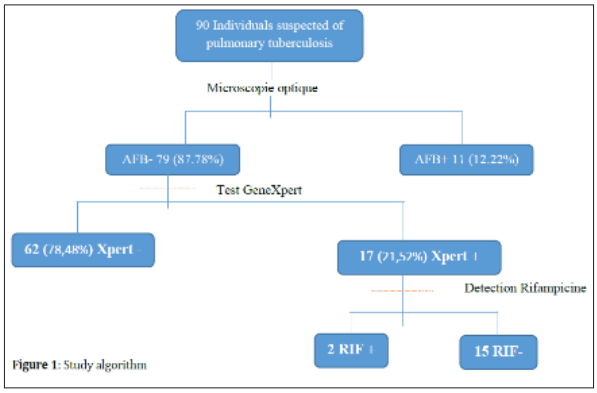

Figure 1: Study Algorithm

HIV Testing

All blood samples were tested by the same kit manufactured by the same company. Three rapid tests, Determine HIV 1/2 test (Alere GmbH, Koln, Germany), Test SD BIOLINE HIV-1/2 3.0(STANDARD DIAGNOSTICS,Republic of Korea) and Test Uni-GoldTM HIV (TRINITY BIOTECH PLC Ire-land) have been used successively for HIV testing. All sam-ples were prepared following the manufacturer instructions.

Ethical clearance was obtained from the CERSSA, institu-tional ethics committee de l'Institut National de Recherche en Science de la Santé (IRSSA) (No. 00000067/DGRST/CERSSA).

Statistical Analysis

We used Epi info 7.2.2 software for the statistical analysis. The P-value of less than 0.05 was considered to indicate sta-tistical significance.

Results

A total of 90 M. tuberculosis-presumptive patients were included in the study (Figure 1). The majority of the study participants were males 69 (76.67%). The mean age was 38.9 ± 12.7. The median was 38 years, the maximum age was 81 years and the minimum of 15 years. Most of the subjects (86.67%) were under >25 years of age.

The microscopic results were represented as follows: 11 (12.22%) had a high bacillary load compared to 79 (87.78%) with a low bacillary load. The overall prevalence of M. tu-berculosis-confirmed cases was 26.67%. Of the 24 (26.67%) M. tuberculosis-confirmed cases, 4 (4.44%, 95% CI 1.22-10.99) were resistant to rifampicin. Rifampicin-resistant M. tuberculosis was observed among HIV seropositive 2 (50%), males 2 (50%), and previously treated tuberculosis patients 2 (50%), although no significant association was found in this study.

Of the 90 individuals, the microscopic results were represented by 11 (12.22%) individuals with a high bacillary load against 79 (87.78%) with a low bacillary load. The GeneXpert technique detected 24 (26.67%) cases of positive GeneXpert showing the presence of Mycobacterium tuberculosis against 66 (73.33%) who did not have mycobacteria.Table1 This ta-ble shows that the cases of chronic cough 17 (45.95%) were more important among the positive cases of GeneXpert.

Table 2 This table shows that the cases of cough 52 (68.42%) were more important among the positive cases of GeneXpert

Table 1: Distribution of Microscopic Results

Variables	AFB-	AFB+	P-value
Demographic characteristic			
Male n (%)	63 (79.75%)	6 (54.55%)	0.1
Symptoms			
Cough	65 (82.28%)	11 (100%)	0.2
Type of cough			
Aigue	37 (56.92%)	2 (43.08%)	0.02
Chronic	28 (18.18%)	9 (81.82%)	
Duration cough			
<2 Weeks	30 (46.15%)	0 (0%)	0.002
>2 Weeks	35 (53.85%)	11 (100%)	
Comorbidities			
HIV	17 (21.52%)	3 (27.27%)	0.7

Table 2: Distribution of Gene Xpert Results

Variables	GeneXpert -	GeneX-pert+	P-value
Demographic characteristic			
Male n (%)	48 (77.42%)	21 (75%)	0.7
Symptoms			
Cough	49 (79.03%)	27 (96.43%)	0.055
Type of cough			
Aigue	30 (61.22%)	9 (33.33%)	0.03
Chronic	19 (38.78%)	18 (66.67%)	
Duration cough			
<2 Weeks	27 (55,10%)	3 (11,11%)	0.0001
>2 Weeks	22 (44,90%)	24 (88,89%)	
Comorbidities			
HIV	12 (60%)	8 (40%)	8 (40%)

AFB and GeneXpert Diagnostic

All sputum samples were processed for smear microscopy to confirm smear negativity for AFB and found negative by ZN staining method. The sputum samples were further pro-cessed for GeneXpert MTB/RIF assay. A known AFB-positive specimen and AFB-negative specimens were also in-cluded to serve as controls for validation of the ZN staining protocol. A total of 90 sputum specimens from suspected TB patients were tested for tuberculosis by AFB test, GeneXpert. 48 (18.39%) cases were detected by AFB test and 62 (23.76%) by GeneXpert.

The type of cough, whether acute or chronic, was associated with AFB - ($p = 0.02$). The duration of cough corresponding to less than 2 weeks was associated with the results of AFB -, while when the duration exceeded 2 weeks was associated with AFB + ($p = 0.002$). The type of cough, whether acute or chronic, was associated with a Genexpert - ($p = 0.03$). The duration of cough corresponding to less than 2 weeks was associated with the results of GeneXpert -, while when the duration exceeded 2 weeks was associated with Genexpert + ($p = 0.0001$).

Discussion

In this study, sputum specimens from presumptive TB cases from Brazzaville were investigated to evaluate the efficiency of GeneXpert MTB/RIF assay against screened conventional ZN staining smear-negative results. Rasool et al, reported that approximately 17% of TB disease transmission is caused by AFB smear microscopy–negative TB suspects and there-fore the risk of disease transmission by AFB-negative cases to healthy individuals could not be ignored [19].

In Republic of Congo, the diagnosis of pulmonary tubercu-losis remains a public health problem in our country, espe-cially in immunocompromised subjects [20,21]. A study car-ried out in Brazzaville, at the Tuberculosis Center revealed that the eligibility criteria were set up in such a way that only cases of patients with therapeutic failure were diagnosed with this new molecular tool, because of reagents in reduced numbers [9]. A selection of patients based on the risk analy-sis could allow a rational use of this test. However, this does not take into account bacteriologically confirmed smear negative cases, which may prove to be geneXpert positive. In addition, resistance to rifampicin is a good predictor of multi resistance. The presence of primary resistance in the population suspected of pulmonary tuberculosis is import-ant in our country (2.4%).

Smear-negative pulmonary TB patients (SNPT) represents between 30% and 60% of all pulmonary TB cases (Luo et al., 2017) and 10–20% of TB transmission at the population level are attributable to SNPT cases [22]. In addition, the high fre-quencies of Smear-negative pulmonary TB patients are ob-served in countries with a high prevalence of HIV infection [23,24]. HIV co-infection, is seen as both a risk factor and an aggravating factor [25]. Of the 90 individuals, the micro-scopic results were represented as follows: 11 (12.22%) had a high bacillary load against 79 (87.78%) had a low bacillary load. On the other hand, GeneXpert has detected Mycobac-terium tuberculosis, the pathogenic agent which confers pul-monary tuberculosis in 24 individuals, ie 26.67%. Okonkwo et al., In a similar study, found a prevalence of 19.8% of MTB detected [26]. This higher prevalence is certainly due to the fact that our study took place in a hospital setting where a high concentration of individuals showing suspected pulmo-nary tuberculosis is found.

The prevalence of HIV in people with pulmonary tubercu-losis was 22.47%. Dagnra et al. revealed in 2010, among 569 patients, 135 (23.7%) were infected with HIV. Mycobacteri-um tuberculosis co-infection is the leading cause of death in people infected with HIV-1. The increase in drug-resistant tuberculosis threatens the global fight against tuberculosis and constitutes a major public health problem [27]. Okem-ba-Okombi et al. in their study at the Brazzaville Tuber-culosis Center observed cases of resistance to Rifampicin (18.02%) [9].

Xpert could greatly reduce the frequency and impact of unnecessary empiric treatment, contact investigation, and housing, providing substantial patient and programmatic benefits if used in management decisions. This is the first study to demonstrate the

potential of GeneXpert M. tuber-culosis/rifampicin to increase case findings, identify drug resistance, and guide treatment of TB in Brazzaville. There is benefit of Gene-Xpert testing in difficult to diagnose pa-tient with sputum smear negative pulmonary tuberculosis. GeneXpert test potentially limit loss to follow up during di-agnostic evaluation of smear negative tuberculosis patients.

AFB smear-negative cases found to have delays in treatment initiation, and especially that there are more and more false negative cases which are detected positive by molecular biology or culture. The GeneXpert MTB/RIF assay (Xpert) must be a novel automated diagnostic tool for tuberculosis and its optimal placement in the healthcare system must be fine de-termined. There is possibility of additional case detection for pulmonary tuberculosis by offering Xpert to smear-negative patients. Patients routinely presenting with symptoms suggestive of PTB with negative smears can thus benefit from the Xpert test which increase pulmonary TB case confirmation. GeneXpert could use for rapid and accurate diagnosis of TB in Republic of Congo, country where TB endemic settings. This will help to detect cases that may have been missed by AFB test and since GeneXpert could detect RR-TB, early en-hance patient treatment and management.

Chronic cough was associated with the presence of Mycobacterium tuberculosis ($p = 0.01$). This has been widely de-scribed in several studies of pulmonary tuberculosis [28-30]. The presence of cough during the clinical examination was also associated with the presence of Mycobacterium tuber-culosis ($p = 0.01$). Cough is a risk factor for pulmonary tu-berculosis and chronic cough is significantly linked to the detection of Mycobacterium tuberculosis [30-32].

The prevalence of HIV in people with pulmonary tuberculo-sis was 22.47%. Mycobacterium tuberculosis co-infection is the leading cause of death in people infected with HIV-1. In our study, HIV status was not associated with the detection of AFB- ($p = 0.7$). Rates of smear-negative pulmonary have been rising in countries with HIV epidemics. The mortali-ty rate among HIV-infected tuberculosis patients is higher than that of uninfected tuberculosis patients, particularly for those with smear-negative pulmonary. Delayed diagno-sis may be an important cause of excess mortality in people living with HIV who have smear-negative pulmonary. In the absence of rapid, simple, and accurate diagnostic tools for smear-negative pulmonary, diagnostic algorithms could be recommended.

Conclusion

Early diagnosis of pulmonary tuberculosis in smear negative pulmonary TB suspects is critical for early initiation of ther-apy. In our Country, GeneXpert assay should be used as a gold standard to diagnose all TB forms. Smear-negative TB represents a large proportion of TB cases in the Republic of Congo, and occurs more often among persons in groups more likely to undergo TB screening. The lower mortality may indicate earlier TB detection, and underscores the need for continued vigilance in screening of high-risk persons. Our study confirms the utility of GeneXpert in the diagnosis of tuberculosis especially smear negative pulmonary tuber-culosis.

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Availability of Data and Materials

All the data are already found in the manuscript and there are no supplementary files. The original data supporting this finding will be available at any time upon request of the cor-responding author.

Disclosure Statement

Authors have seen and approved the manuscript being submitted and there is no conflict of interest. We warrant that the article is the Authors' original work. This research has not been submitted for publication nor has it been published in whole or in part elsewhere. Authors listed on the title page have contributed significantly to the work, have read the manuscript, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission to the Journal for publication

Notes

Ethics Approval and Consent to Participate

The study was conducted in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 2000. This study was reviewed and approved by the Comité éthique de la Recherche en Sciences de la Santé (CERSSA). Since this was a research study, written informed consent was request-ed for study participation from every participant. None of the data can be linked to individuals and no patient identi-fiers were used. Confidentiality of the information collected was maintained by omitting their name and other personal identifiers from extraction sheet. The study did not require collection of additional patient samples or performance of any test additional to routine patient care procedures so the study did not constitute a risk for the patients. The study was conducted inside an ongoing research project aiming at the development of a comprehensive clinical approach towards the diagnosis of pulmonary tuberculosis in Republic of Con-go.

Consent for Publication

Not applicable.

Conflicts of Interest

The authors declare that there are no conflicts of interest re-garding the publication of this paper. There are no conflicts of interest of any kind, including any relationship or dual interest, financial or otherwise, that may affect professional judgment in relation to this article.

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