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Missed Opportunities for Diagnosing Bacilliferous Pulmonary Tuberculosis by Optical Microscopy versus GeneXpert MTB/RIF in Endemic Areas

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Abstract

Objective: To assess the missed opportunities from the diagnosis of bacilliferous pulmonary tuberculosis by optical microscopy compared to GeneXpert MTB/RIF between 2015 and 2019. **Methods**: This is a retrospective analysis of the diagnostic results of bacilliferous pulmonary tuberculosis in patients suspected of pulmonary tuberculosis at their first episode during the period. GeneXpert MTB/RIF (GeneXpert) and optical microscopy (OM) after Ziehl-Neelsen stained smear were performed on each patient's sputum or gastric tubing fluid sample. **Results**: Among 341 patients suspected of pulmonary tuberculosis, 229 patients were declared bacilliferous tuberculosis by the two tests (67%), 220 patients by GeneXpert and 95 patients by OM, *i.e.* 64.5% versus 28% ($p < 0.0001$, OR = 7.99). The OM ignored 134 tuberculosis patients detected by the GeneXpert (61%), *i.e.* 58.5% of the positive cases detected by the two tests (134/229 patients) and 39.3% of the patients suspected of tuberculosis (134/341 patients). On the other hand, among 95 patients declared positive by OM, the GeneXpert ignored 9 (9.5%), *i.e.* 4% of all the positive cases detected by the two diagnostic tests (9/229 patients) and 3% of the patients suspected of tuberculosis (9/341 patients). The differences observed between the results of the two tests were statistically significant at the 5% threshold ($p < 0.0001$). **Conclusion**: This study reveals missed diagnostic opportunities for bacilliferous pulmonary mycobacteriosis, statistically significant with optical microscopy than GeneXpert. The GeneXpert/optical microscopy couple could be a good contribution to the strategies for the elimi-

nation of pulmonary tuberculosis in sub-Saharan Africa.

Keywords

Bacilliferous Pulmonary Tuberculosis, Missed Opportunity, GeneXpert MTB/RIF, Optical Microscopy

1. Introduction

In Côte d'Ivoire, the Tuberculosis burden remains high despite a drop in its incidence which fell from 367 cases in 2000, to 148 cases in 2017, 142 cases in 2018 and 137 cases per 100,000 in 2019 according to the World Health Organization (WHO) [1]. In order to respond to current global priorities aimed at improving the early detection of cases, including those of multidrug-resistant tuberculosis (MDR-TB), the WHO approved in 2010 GeneXpert, which is a fully automated molecular test to detect *Mycobacterium tuberculosis* (MTB) and its resistance to rifampicin (RIF) directly in clinical samples [2]. This GeneXpert system is currently considered an important advance in the fight against tuberculosis because of its diagnostic efficiency, its less restrictive use in the field compared to other conventional diagnostic methods, and its capacity for rapid diagnosis of a large number of biological fluids, thus justifying its importance in public health [3] [4]. Therefore, to strengthen its diagnostic strategies, the National Program for the Fight against Tuberculosis in Côte d'Ivoire (PNLT-ci) adopted 2013 the GeneXpert MTB/RIF system for the rapid diagnosis of tuberculosis, but limiting its indications to two categories of patients: on one hand, patients at high risk of multi-drug resistant tuberculosis "patients eligible for retreatment, MDR-TB contacts-patients with positive smears after the intensive phase of the first-line regimen" and those whose bacteriological diagnosis by microscopy is made difficult due to various reasons "children under 14 years old, people living with HIV screened positive for tuberculosis and cases of suspected pulmonary tuberculosis by microscopy negative satisfying national guidelines" [5]. With the aim of making available scientific evidence about the importance of GeneXpert in routine, We carried out this study with the objective of evaluating the missed opportunities in the diagnosis of bacilliferous pulmonary tuberculosis by optical microscopy compared to GeneXpert MTB/RIF.

2. Material and Methods

- **Study design**

This study was part of a pilot project for the molecular diagnosis of tuberculosis using the GeneXpert MTB/RIF initiated by the PNLT-ci in 2015. This was a cross-sectional study carried out from January 1, 2015, to December 31, 2019, on samples of sputum or gastric tubing fluid at the Anti-Tuberculosis Center (ATC) in the city of Bouaké. It is a national reference center, equipped with human resources and adequate equipment to ensure the screening and management of

tuberculosis during routine consultations. The city of Bouaké is located in the center of the Ivory Coast, 150 km from Yamoussoukro, the administrative capital of the country and 300 km from Abidjan. Included were patients suspected of pulmonary tuberculosis, at their first stage of pulmonary tuberculosis, in whom the GeneXpert MTB/RIF test and optical microscopy of Ziehl-Neelsen (ZN)-stained smears were performed on each sputum sample or gastric tube fluid looking for bacilliferous tuberculosis. On the other hand, patients already under anti-tuberculosis treatment and patients who had recently taken antibiotics likely to negatively affect the detection of Mycobacteria of the tuberculosis complex (amoxicillin-clavulanic acid, fluoroquinolones, aminoglycosides) were not included in the study. The pathological products (sputum and gastric tubing fluids) were collected from patients admitted to the pulmonology and pediatric departments of the Bouaké University Teaching Hospital and the outpatient consultation at the Bouaké ATC. Bacilliferous tuberculosis was defined by the positivity of GeneXpert and/or the presence of acid-fast bacilli (AFB) on Ziehl-stained smears by optical microscopy. No cultures were performed on biological fluids.

- **GeneXpert MTB/RIF molecular test**

Each sample was immediately tested according to the manufacturer's instructions by the GX system based on real-time polymerase chain reaction (PCR) which detects MTBC DNA and looks for mutations that confer resistance to RIF. To detect strains of the MTB complex and mutations associated with RIF resistance, the central 81 bp target region of the rpoB gene is amplified and coupled with five molecular beacons or probes (A, B, C, D, E type "beacons") that overlap along the target sequence [6] [7] [8].

- **Bacilloscopy or search for acid-fast bacilli under an optical microscope**

Smears were prepared from the pellets for each sample, then stained by the Ziehl-Neelsen method and observed under an optical microscope to look for the presence of acid-fast bacilli (AFB). Smears showing red-colored bacilli were considered positive for the diagnosis of tuberculosis [9].

- **Data analysis and ethical considerations**

The parameters studied related to the demographic profile of the patients included (age, sex, comorbidities), the diagnostic efficacy of the GeneXpert MTB/RIF test and optical microscopy (sensitivity and specificity). Data analysis was performed using Epi Info 7.2 software. Data comparison was performed using Pearson's "chi-square" test with a significance threshold of 5%. The confidentiality of patient data was ensured by assigning an anonymous number to each survey form with the collaboration of the survey center's social service. The study obtained investigation authorization from the scientific medical committee of the Bouaké University Hospital.

3. Results

- **Patient demographics (Table 1)**

Table 1. Profile of study patients.

Parameters	Number N = 341	Percentage %
Sex		
Male	201	59
Female	140	41
Age (yrs)		
[5 - 9]	16	05
[10 - 19]	31	09
[20 - 49]	203	60
[50 - 60]	39	11
[61 - 83]	52	15
Serology HIV		
Positive	94	28
Negative	15	04
Not known	232	68
Confirmed smoking	66	19
Diabetes	01	0.3

During the study period, 341 patients were included in the study for suspected pulmonary tuberculosis. They were mostly men with an M/F sex ratio of 1.4. The mean age was 39.68 ± 18.06 years [extremes of 5 and 83 years]. AIDS, smoking and diabetes accounted for 28%, 19% and 0.3% of patients respectively.

- **Frequency of bacilliferous pulmonary tuberculosis**

Among the 341 patients suspected of pulmonary tuberculosis, 220 patients were positive according to the GeneXpert test (64.5%) and 95 patients by optical microscopy (28%). Positive concordance of the two tests (GeneXpert and optical microscopy) was observed in 86 patients (25%). Overall, 229 patients (67%) tested positive, thus benefiting from antituberculosis treatment (Table 2).

- **Analytical data**

Table 1 shows the performance of the two diagnostic tests for pulmonary tuberculosis. Among the 341 patients suspected of tuberculosis, 229 patients were confirmed to have tuberculosis by both tests (67%), 220 patients by GeneXpert and 95 patients by optical microscopy, *i.e.* 64.5% versus 28% ($p < 0.0001$, OR = 7.99). Thus, optical microscopy ignored 134 tuberculosis patients detected by the GeneXpert (61%), *i.e.* 58.5% of the positive cases detected by the two tests together (134/229 patients) and 39.3% of the patients suspected of tuberculosis (134/341 patients). These 134 patients would not have received anti-tuberculosis treatment if they had not been tested with GeneXpert. Conversely, among 95 patients declared positive by optical microscopy, the GeneXpert ignored 9 (9.5%), *i.e.* 4% of all positive cases detected by the two diagnostic tests (9/229 patients) and 3% of patients suspected of tuberculosis (9/341 patients). These patients took anti-TB treatment, based on optical microscopy. The differences observed between the results of the two tests were statistically significant at the 5% threshold

Table 2. Microscopy and GeneXpert in the diagnosis of bacilliferous tuberculosis.

	GeneXpert positive N = 220	GeneXpert negative N = 121	Total N = 341	p	OR	IC
Ziehl-Neelsen positive	86	9	95	<0.0001	7.99	3.85 - 16.6
Ziehl-Neelsen négative	134	112	246			

Table 3. Limits of optical microscopy and GeneXpert in the diagnosis of bacilliferous tuberculosis.

Limits of the two tests in relation to	GeneXpert	Microscopy	Difference	p	OR	IC
Opposite test positivity	9/95 (9.5%)	134/220 (61%)	51.5%	<0.0001	7.99	3.85 - 16.6
Both tests Positivity	9/229 (4%)	134/229 (58.5%)	54.5%	<0.0001	0.03	0.01 - 0.06
Tuberculosis suspected cases	9/341 (3%)	134/341 (39%)	36.3%	<0.0001	0.06	0.03 - 0.12

(**Table 3**).

4. Discussion

In sub-Saharan Africa, the diagnosis of bacilliferous pulmonary tuberculosis has long relied routinely on optical microscopy of pathological products, in particular sputum and gastric tubing fluids. Culture, which remains the reference microbiological examination, is however little practiced due to material and technical constraints, in particular the long delay in reporting results of approximately 2 to 3 weeks [10] [11] [12]. Today, the recent advent and use of new molecular biology methods with GeneXpert, whose diagnostic performance for the detection of *Mycobaterium tuberculosis* is unquestioned [6] [13] [14] [15] now make it possible, in addition to optical microscopy, to establish the diagnosis of tuberculosis earlier and to initiate specific treatment. In this study, among 341 patients suspected of pulmonary tuberculosis, 229 patients were confirmed to have bacilliferous tuberculosis by both tests (67%), including 220 patients by GeneXpert and 95 patients by optical microscopy, *i.e.* 64.5% versus 28% ($p < 0.0001$) with positive concordance of the two tests (GeneXpert and optical microscopy) in 86 patients (25%).

This study confirms the diagnostic performance of GeneXpert and bacilloscopy in identifying missed opportunities for the diagnosis of bacilliferous pulmonary tuberculosis in an urban reference center in Côte d'Ivoire. It reveals the frequency of tuberculosis in the communities in Côte d'Ivoire regardless of gender or age [1]. Its association with AIDS (28%) and smoking (19%) calls for strengthening the means of awareness and prevention, so as to reduce the inci-

dence of these comorbidities.

Among the patients suspected of pulmonary tuberculosis previously selected based on epidemiological, clinical and radiographic arguments, the majority were confirmed by both tests (67%), including 64.5% by GeneXpert but 28% by optical microscopy. Thus, optical microscopy ignored 61% of patients detected by GeneXpert, *i.e.* 58.5% of positive cases by both tests combined. Initially, one could evoke, among these cases ignored by optical microscopy, false positives to GeneXpert by the detection of DNA of non-viable bacilli in former tuberculosis patients [16]-[20]. However, this study did not include patients with history of anti-tuberculosis treatment or active tuberculosis during treatment. Furthermore, according to the available data, no cross-reactivity has been reported between GeneXpert MTB/RIF and several species of bacteria, viruses, fungi or mycobacteria other than tuberculosis. This test should therefore not be the source of false positive results [21] [22].

The performance of bacilloscopy is less effective than that of GeneXpert. It could depend, on one hand, on the quality of the samples taken and the appropriate sampling conditions, and on the other hand on the detection threshold. Indeed, bacilloscopy is generally positive only from the threshold of 10,000 bacteria per milliliter of sputum: below this threshold, AFB would not be detected [23]. It is therefore these pauci-bacillary patients who escaped the bacilloscopy that the GeneXpert probably detected. With bacilloscopy performed routinely, these 134 ignored bacilliferous patients would therefore be potential sources of dissemination of tuberculosis to those around them, therefore maintaining the continuity of the tuberculosis endemic.

Conversely, the GeneXpert is not fully efficient. Indeed, among 95 patients declared positive by bacilloscopy, he ignored 9 patients (9.5%), which represents 4% of all positive cases detected by the two diagnostic tests and 3% of patients suspected of pulmonary tuberculosis. It should be recalled that optical microscopy has the particularity of detecting mycobacteria in the form of AFB without any specification of the species, whereas GeneXpert only detects *Mycobacterium tuberculosis*. Thus, the AFB detected in patients ignored by the GeneXpert could be *non-tuberculosis mycobacteria.* Other examinations, such as culture of mycobacteria on Lowenstein-Jensen or Bactec media, are necessary to determine the species of these mycobacteria not recognized by GeneXpert. Thus, the sole use of GeneXpert for the diagnosis of tuberculosis could expose to the selection and emergence of non-tuberculosis mycobacteria species in regions where tuberculosis is hyper-endemic.

5. Conclusion

This study reveals missed opportunities for the diagnosis of bacilliferous pulmonary mycobacteriosis. These missed opportunities statistically greater with optical microscopy than with GeneXpert, probably explain the persistence of tuberculosis endemic in countries where optical microscopy has remained the

routine microbiological examination. The diagnostic efficacy of the GeneXpert MTB/RIF no longer needs to be demonstrated compared to optical microscopy. However, the GeneXpert MTB/RIF ignores a small proportion of AFB. Thus, these two "GeneXpert couple with optical microscopy" examinations could be a good contribution to the strategies for the elimination of pulmonary tuberculosis in sub-Saharan Africa.

Contribution of the Authors

AHV and AYT designed and planned the study. NDO, YL and YMT collected the data. AYT, NDO, YL, MP and AHV analyzed the data and wrote the manuscript. All authors have read and approved the final version.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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