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1 **Sampling strategy for bacteriological diagnosis of intrathoracic tuberculosis**

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31 **Abstract**

32 *Background.*

33 Pulmonary tuberculosis (TB) is the most frequent site of TB and the one leading its spread worldwide.
34 Multiple specimens are commonly collected for TB diagnosis including those requiring invasive
35 procedures. This study aimed to review the sampling strategy for the microbiological diagnosis of
36 pulmonary TB.

37 *Methods.*

38 A retrospective analysis of collected samples from September 1st 2014 to May 1st 2016 in the
39 Bacteriology laboratory of Pitié-Salpêtrière Hospital (Paris, France) was performed. All the samples
40 collected in patients aged over 18 years for the bacteriological diagnosis of pulmonary TB were included.

41 *Results.*

42 A total of 6,267 samples were collected in 2,187 patients. One hundred and twenty-six patients (6%)
43 had a culture confirmed pulmonary TB. Among them, multiple sputum collections were sufficient for TB
44 diagnosis in 63.5%, gastric lavages permitted to avoid bronchoscopy in only 7.1%, and bronchoscopy
45 was necessary in 29.4%. The culture positivity of sputa (8.6%) was higher than that of bronchial
46 aspirations (3.1%), bronchiolo-alveolar lavages (BAL) (2.3%) or gastric lavages (4.8%) ($p < 0.001$). From
47 its 70.0% theoretical PPV value, the 46.1% selection in bronchial aspirations allocated to molecular test
48 increased PPV up to 88.9%.

49 *Conclusions.*

50 Based on our data, we suggest to collect sputum consistently. If smear negative a bronchoscopy should
51 be performed and molecular diagnosis be performed on a subset of bronchial aspirations based on
52 expertise of the bronchoscopist.

53

54

55

56 **1. Introduction**

57 The global threat of tuberculosis (TB) remains undiminished with the WHO estimating there were 10
58 million incident cases worldwide in 2018 [1]. In 2020, France is a low TB incidence country [2] but TB
59 remains a significant public health problem, particularly in some geographic areas [3–7] and susceptible
60 populations [4,6,8]. Pulmonary TB includes pulmonary, and mediastinal lymphadenopathy involvements
61 [9]. It constitutes about 80% of total TB cases [1] and is the main cause of bacilli spread in the
62 environment [10–12]; thus driving TB epidemic worldwide. The definite diagnosis of active pulmonary
63 TB requires the culture isolation of *Mycobacterium tuberculosis* complex (M. tb) from respiratory sample
64 [13]. Molecular tests are increasingly used in TB diagnosis, allowing a significant reduction of its delay
65 compared to culture [14–16] and early treatment initiation. The positive predictive value (PPV) of
66 molecular tests directly performed on a specimen is correlated to its expected culture positivity,
67 depending on the local epidemiology of TB [17]. Significant attention must be paid in the choice of the
68 specimen allocated to molecular tests among the multiple ones which are sampled for the diagnosis of
69 pulmonary TB, particularly in a low TB incidence setting such as France since PPV's specimens are
70 expected to be lower than in high TB incidence countries. Multiple sampling [18–20] are provided by a
71 non-invasive procedure like sputum collection eventually after induction [21] or, if necessary, by an
72 invasive procedure such as bronchoscopy or gastric lavages. Post-bronchoscopy sputum specimens
73 have to be collected from all adults with suspected pulmonary TB who undergo bronchoscopy [21,22].
74 Among per-bronchoscopy sampling procedures, endobronchial ultrasound transbronchial needle
75 aspiration (TBNA) has recently demonstrated interesting safety and sensitivity in *M. tb* isolation in hilar
76 and mediastinal lymphadenopathy [23–29]. A reasoned use of invasive procedures requires knowing
77 the individual profitability of their relative sample in the microbiological diagnosis of TB, compared to
78 non-invasive, inexpensive, and rapid sampling such as sputum with or without induction.
79 This study aimed to review the bacteriological diagnosis of pulmonary TB and to assess the contribution
80 of invasive procedure to diagnosis, in a real life context.

81

82 **2. Materials and methods**

83 *2.1. Study population*

84 We performed a retrospective cohort study including all patients over 18 years of age who were sampled
85 for bacteriological diagnosis of TB, hospitalized in Pitié-Salpêtrière Hospital (Paris, France), between
86 September 1st, 2014 and May 1st, 2016.

87 *2.2. Specimen*

88 Microbiological results from sputum, gastric lavages, bronchial aspirations, bronchiolo-alveolar lavage
89 (BAL), bronchial biopsies and TBNA were extracted from the laboratory management software GLIMS.
90 Post-bronchoscopy sputum was defined as sputum collected during the three days following a
91 bronchoscopy.

92 In the pulmonary TB diagnosis approach, multiple sputa were collected in first line. In sputum scarce or
93 when 3 sputa were smear negative, an invasive procedure was performed *i.e.* bronchoscopy or gastric
94 lavages. When gastric lavage was considered, its place in the local approach was always in first line,
95 *i.e.* before bronchoscopy. All specimens were categorized as invasive except pre-bronchoscopy sputa
96 which were not collected within the 3 days following bronchoscopy, named pre-bronchoscopy sputa.

97 *2.3. Statistics*

98 The proportions were compared with the bilateral Fisher exact test calculated using GraphPad prism
99 software. All hypotheses were tested at alpha risk of 0.05.

100

101 *2.4 Molecular test's PPV*

102 The molecular test used was the DX MTB ASSAY (PCR) during the study period. According to local
103 diagnostic strategy set up with the bronchoscopy ward, this test was preferentially done on
104 bronchoscopy specimens. This strategy was supported by the fact that patients undergoing
105 bronchoscopy have by definition smear negative specimens and are at high risk of TB and are evaluated
106 by a small number of doctors that usually have a computed tomography (CT) scan to help them. As a
107 consequence, the doctor performing the bronchoscopy was considered as the best for deciding if a PCR
108 needed to be performed for TB diagnosis. We compared the actual PPV in our population to the
109 theoretical PPV if the PCR had been performed on all bronchial specimens. According to the literature
110 the sensitivity of the DX MTB ASSAY is 58% on smear negative specimens and specificity 99% [30].

111 The prevalence corresponding to the proportion of culture positivity, the specimens PPV were calculated
112 by the following formula: $PPV = \frac{Se \times Prevalence}{[Se \times Prevalence + (1 - Prevalence) \times (1 - Sp)]}$.

113

114 **3. Results**

115 *3.1. Study population and diagnostic procedure*

116 From September 1st, 2014 to May 1st, 2016, a total of 2,187 patients benefited from at least one
117 microbiological sampling for pulmonary TB suspicion and were retrospectively included (Figure 1).
118 Among invasive procedures, bronchoscopy was performed in 1,317 patients (60.2%). A total of 126
119 patients (5.8%) had a culture confirmed pulmonary TB. Among them, pre-bronchoscopy sputum was
120 sufficient to diagnose their TB in 80 patients (63.5%), whereas the diagnosis required specimens
121 collected after an invasive procedure for the remaining 46 patients (36.5%), (gastric lavage in 9/46
122 patients (19.5%), bronchoscopy in 37/46 patients (80.4%)) (Figure 2). Among these 37 TB patients
123 benefiting from bronchoscopy, the majority had culture positivity from bronchial aspirations 24/37
124 (64.9%), followed by BAL 6/37 (16.2%), post-bronchoscopy sputum 5/37 (13.5%) and TBNA 2/37
125 (5.4%).

126 Among patients who had *M. tb* positive culture from bronchial (n=1) or TBNA (n=4) biopsies, these
127 invasive samples were the only ones allowing the diagnosis in 0.0% and 50.0% of patients, respectively.
128 In the 99 patients with at least one culture positive sputum, more than one sputum collection was
129 necessary to diagnose TB in 28.3% and more than 3 in 10.1% (Table 2).

130

131 *3.2 Specimen's smear and culture positivity for Mycobacterium tuberculosis*

132 A total of 6,267 specimens were collected and analysed. Among them, those exhibiting the highest
133 proportion of *M. tb* culture positivity were in decreasing order, bronchial biopsies, TBNA, sputa, gastric
134 lavages, bronchial aspirations, and finally BAL, which exhibited the lowest proportion of culture positivity.
135 The proportion of culture positivity in sputum was higher than (Table 1) in bronchial aspirations (8.6%
136 vs. 3.1% $p < 0.0001$), BAL (8.6% vs. 2.3% $p < 0.0001$) or gastric lavages (8.6% vs. 4.8% $p = 0.0004$). The
137 proportion of culture positivity in TBNA was higher than in bronchial aspirations (13.3% vs. 3.1%
138 $p < 0.0001$) and BAL (13.3% vs. 2.3% $p < 0.0001$), but not compared to sputum (13.3% vs. 8.6% $p = 0.09$).

139 Specimens exhibiting the highest proportion of smear positivity were in decreasing order, sputa, TBNA,
140 gastric lavage, BAL, bronchial aspiration and finally bronchial biopsies (Table 1). The smear positivity
141 was higher in sputum than in gastric lavage (5.2% vs. 2.8% p=0.007), bronchial aspirations (5.2% vs.
142 0.3% p<0.0001) and BAL (5.2% vs. 0.5% p<0.0001). The smear positivity in TBNA was higher than in
143 bronchial aspirations (3.3% vs. 0.3% p<0.0001), and BAL (3.3% vs. 0.5% p<0.0001).

144

145 *3.3 Theoretical and actual PPV values of molecular test directly performed on smear negative bronchial* 146 *aspirations*

147 PCR was proposed on smear negative bronchial aspirations of patients with a high suspicion of
148 pulmonary tuberculosis as assessed by the bronchoscopist based on the analysis of the clinical file and
149 the CT scan of the patient. This selection retained 46% of all bronchial aspirations for PCR (627/1354).
150 Among those, the proportion of culture positivity was significantly higher than among those not retained
151 for molecular testing (4.62% vs 1.64%, p=0.002). Consequently, the positive predictive value (PPV) of
152 molecular testing was high (88.9%) to be compared with a 70% theoretical PPV if molecular testing had
153 been performed on all bronchial aspirations without the selection performed by the bronchoscopist
154 (Table 3).

155

156 **4. Discussion**

157 We present a large overview of the global interest of the different type of specimens in the bacteriological
158 diagnosis of pulmonary TB. This study was performed in a reference hospital located in a TB hot spot
159 region of France [3–7], permitting an overview of more than 6,000 specimens collected in a low TB
160 incidence country. Globally, the specimen showing the main utility in pulmonary TB diagnosis is sputum
161 in both culture and smear positivity; the multiple collection of this specimen appeared sufficient to
162 confirm the diagnosis in two-thirds of patients. As in other studies [18,19], we showed a particular
163 interest to collect multiple sputum to increase diagnosis accuracy. In the last third, only 20% obtained
164 their diagnosis by gastric lavages whereas the others required specimens sampled during or after
165 bronchoscopy. Among the different samples categorized as invasive in this study, post-bronchoscopy
166 sputum which is the easiest to sample should be systematically realized just after or the day after the
167 bronchoscopy and simply repeated, showed, in this study, a particular interest with a high proportion of
168 culture and smear positivity.

169 These excellent diagnostic performances of sputa were obtained in our centre without having optimized
170 the pre-analytical procedures. Some interventions have demonstrated that the sensitivity of sputa can
171 be further increased. A particular attention should be paid to the sputum quality and quantity [21,31].
172 Notably, a sputum volume over 4 [32] or 5 mL [33] increases significantly smear positivity. In a recent
173 study, Datta *et al.* showed that two simple interventions increased the diagnosis performance of
174 microbiological testing. Pooling of sputum samples collected over a period of several hours increased
175 the diagnosis performance of smear microscopy 1.6 times and of culture 1.7 times, compared with
176 collection of a single time point or spot samples and the delivery of standardized instruction before the
177 provision of sputum samples increased the performance of smear microscopy 1.4 times compared to
178 the standard of care [34].

179 We suggest that gastric lavage should be avoided in practice since (i) this invasive procedure is
180 particularly uncomfortable, (ii) it has to be repeated on several mornings and (iii) it rarely permits to avoid
181 bronchoscopy [35] (only 20% of our TB patients). Moreover, whereas bronchoscopy allows diagnosing
182 several different diseases, gastric lavages are limited to TB. Since the superiority of bronchoscopy
183 compared to multiple gastric lavages is also supported by other studies [35,36], we suggest to perform
184 bronchoscopy immediately after three smear negative sputa. In this relatively infrequent situation when
185 bronchoscopy is necessary, we advise to collect widely all available specimens in order to benefit the
186 most from invasive procedure utility in pulmonary TB diagnosis. Neither TBNA nor BAL collections
187 worsen respiratory failure compared to patients receiving bronchoscopy without these samplings
188 [24,37]. Nonetheless, contrary to BAL, TBNA procedure should be limited to patients with mediastinal
189 or hilar lymphadenopathy visible in thoracic CT scan and accessible to endo-bronchial ultrasound-
190 guided (EBUS) TBNA. In this last situation TBNA provides valuable support in the diagnosis of
191 pulmonary TB [23–29], particularly in necrotic lymph nodes [29,38] and when ultrasound findings reveal
192 clear boundaries and calcifications [28]. Above all, post-bronchoscopy sputum should be systematically
193 collected after bronchoscopy each time BAL is performed, in view of its utility highlighted in our study
194 and in others [39,40], to benefit to the full extent of the bronchoscopy.

195 Molecular tests are increasingly used in TB diagnosis, allowing a significant reduction of delay compared
196 to culture [14–16]. Their main utility is in smear negative specimens which correspond to the most
197 difficult to diagnose TB cases. However, the PPV of molecular testing is limited in this situation by the
198 very small proportion of true positives, *i.e.* culture positive specimens. To help physician pre-test scores

199 would be useful. Such scores have mainly been assessed in extra-pulmonary TB since molecular
200 diagnosis accuracy is particularly affected by their low bacillary load. The utility of this type of score was
201 confirmed repeatedly in TB meningitis (TBM) [41,42], and was recently included in a consensual TBM
202 diagnosis classification [43]. An equivalent score would also be helpful for pulmonary TB; it could be
203 based on those validated to determine the need of early respiratory isolation before *M. tb* isolation
204 [44,45]. In this study rather than using a score, we decided to rely on a small team of trained clinicians,
205 the bronchoscopy team. The advantage of this organization is that this small team it is trained for difficult
206 diagnoses and that they usually have extensive imaging. This organization reduced by approximately
207 50% the number of bronchial aspirations to be submitted for molecular testing and increased its PPV
208 from 70 to 89%. On the other hand, this reduction missed 29% of culture positive bronchial aspirations
209 (12/41) that could have been diagnosed earlier if they had been subjected to molecular testing. In
210 conclusion, regarding molecular test's contribution to TB diagnosis, we propose that until a validated
211 score is available, the indication of molecular testing relies on a small team having expertise and
212 complete medical and radiological information such as the bronchoscopy team in our hospital.
213 Our study was limited by the lack of clinical and radiological detailed information, which did not allow us
214 to grade the pre-test probability of pulmonary TB. Notably, the diagnosis accuracy of biopsies was hard
215 to assess since, contrary to BAL or bronchial aspirations, which were systematically performed during
216 bronchoscopy, they were sampled only in specific patients with radiologic or macroscopic arguments
217 for accessible lymphadenopathy or bronchial TB involvements. This real life study is naturally limited by
218 the fact that diagnosis procedure was not prospectively controlled even if included patient's diagnosis
219 followed the same approach. An explanation of the sputum highest accuracy in TB diagnosis could be
220 that sputum was used for the easiest to diagnose cases that harbour the highest bacillary loads.
221 Nonetheless, we confirmed the importance of post-bronchoscopy sputa whereas in our study those
222 patients had pauci-bacillary TB.

223 **5. Conclusion**

224 This study confirms the higher accuracy of sputum in the microbiological diagnosis of pulmonary TB
225 compared to invasive specimens in our context of low TB incidence. Based on our data, we suggest
226 collecting sputum consistently, and then if both smear and molecular test remain negative,
227 bronchoscopy should be proposed not forgetting post-bronchoscopy sputa. Molecular testing can be
228 performed following indication by the bronchoscopist.

229

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233

234 **Disclosure of interest**

235 The authors declare that they have no competing interest.

236

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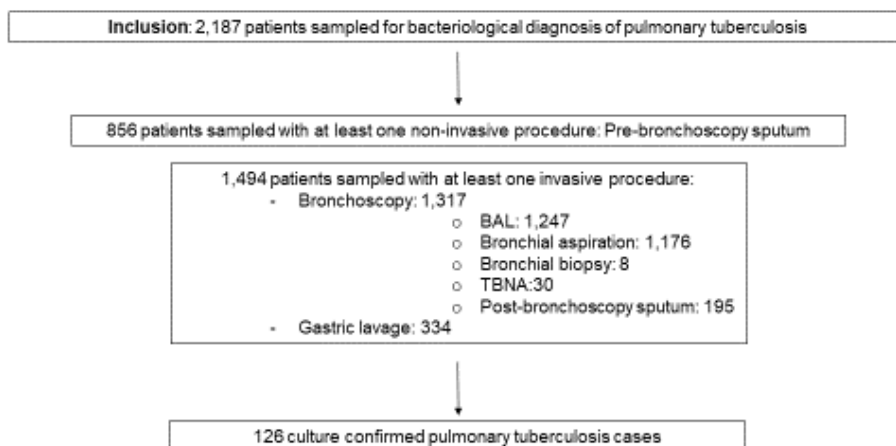
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382 **Figures**



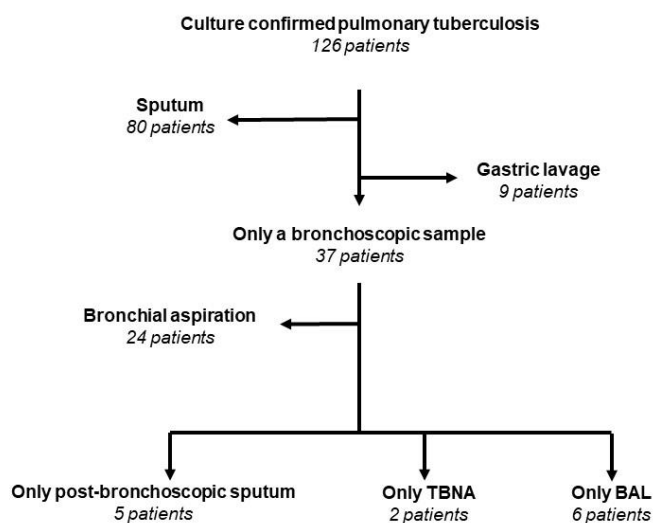
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384 Figure 1: Flow chart of the study design

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389 BAL: Bronchiolo-alveolar lavage

390 TBNA: Transbronchial needle aspiration

391 Figure 2: Distribution of *M. tuberculosis* complex positive culture specimens leading to 126 pulmonary

392 tuberculosis diagnoses

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397 **Tables**

398 Table 1: Bacteriological results of the 6,267 specimens collected from September 1st, 2014 to May 1st,
 399 2016 in patients with suspicion of pulmonary TB.

Type of specimens	n	<i>M. tuberculosis</i> culture- positive samples N (%) [*]	Smear-positive samples N (%) [*]
All sputum	2,591	222 (8.6%)	136 (5.2%)
Pre bronchoscopy sputum	2,018	169 (8.4%)	104 (5.1%)
Post bronchoscopy sputum	573	53 (9.2%)	32 (5.6%)
Gastric lavage	848	41 (4.8%)	24 (2.8%)
Bronchial aspiration	1,358	42 (3.1%)	4 (0.3%)
BAL	1,433	33 (2.3%)	7 (0.5%)
TBNA	30	4 (13.3%)	1 (3.3%)
Bronchial biopsy	7	1 (14.3%)	0 (0.0%)
Total	6,267	343	172

400 ^{*}percentage among the type of sample

401 BAL: Bronchiolo-alveolar lavage

402 TBNA: Transbronchial needle aspiration

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406 Table 2: Individual sputum contribution in the microbiological diagnosis of tuberculosis

Sputum	Patients with at least one culture positive sputum	The first is sufficient	The second is necessary	The third is necessary	More than 3 are necessary
All	99	71 (71.7%)	12 (12.1%)	6 (6.1%)	10 (10.1%)
Pre-bronchoscopy	81	61 (75.3%)	6 (7.4%)	5 (6.2%)	9 (11.1%)
Post-bronchoscopy	18	10 (55.6%)	6 (33.1%)	1 (5.6%)	1 (5.6%)

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412 Table 3: Proportion of culture positivity theoretical and actual PPV of molecular tests directly performed
413 on smear negative bronchial aspirations

Smear negative bronchial aspirations	
Specimens: n	1,354
Culture positivity: n (%)	38 (2.8%)
Expected PPV*: %	70.0%
PCR performed: n (%)	624 (46.1%)
Observed PPV: %	88.9%

414 PPV: Positive predictive value

415 * According to [30]

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