Shortening Isolation of Patients With Suspected Tuberculosis by Using Polymerase Chain Reaction Analysis: A Nationwide Cross-sectional Study

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Background. Isolation of patients suspected for pulmonary tuberculosis is guided by serial sputum smears. This can result in isolation for days for patients with noncontagious tuberculosis. To determine whether a single sample negative for Mycobacterium tuberculosis complex at polymerase chain reaction (PCR) can guide isolation.

Methods. We retrospectively evaluated sputum samples analyzed for Mycobacterium tuberculosis complex at the International Reference Laboratory of Mycobacteriology, Copenhagen, Denmark in 2002–2011. We selected culture-confirmed tuberculosis cases with ≥3 samples within 14 days before or after the initial culture-positive sample. We repeated the process for those with ≥2 samples within 28 days. The primary outcome was PCR-negative, smear-positive patients.

Results. We included 53,533 sputum samples from 20,928 individuals; 1,636 had culture-confirmed tuberculosis. Of these, 856 had ≥3 sputum samples analyzed within the 28 days, and 482 had ≥1 PCR result. Nine patients (2.5% of smear-positive patients) were smear positive/PCR negative; 8 of the 9 had a smear-positive result in only 1 of 3 samples, and 5 had a low smear grade. Of 722 patients with 2 samples, 7 (1.3% of smear-positive patients) were smear positive/PCR negative. Overall, none were smear positive for the sample that produced the negative PCR result.

Conclusions. Primary PCR identified >97% of serial smear-positive cases. The majority of the missed cases had low-grade smears. Nevertheless, the occurrence of smear-positive/PCR-negative cases underlines the importance of increasing the quantity and quality of samples. Moreover, it is important that samples analyzed with PCR are cultured, owing to higher-sensitivity drug susceptibility testing, differential diagnosis, and surveillance.

Keywords. tuberculosis; nucleic acid amplification; polymerase chain reaction; respiratory isolation; in-hospital transmission.
samples [4], because the diagnostic benefit of a third sample is limited [8–11]. In the second scenario, smear status is not exhaustive and unambiguous, because smear microscopy does not differentiate live from dead bacilli.

Recommendations differ internationally but generally include clinical improvement, reduced cough, and/or effective treatment for 2 weeks, effectively eliminating the risk of transmission [12, 13]. In Denmark, tuberculosis guidelines recommend isolation in single-patient rooms for presumed drug-sensitive pulmonary tuberculosis, while suspected multidrug-resistant tuberculosis cases are isolated in negative-pressure rooms. Danish recommendations for discontinuation of isolation include 3 consecutive negative smears, or 2 weeks of effective antituberculosis treatment [14]. Because prolonged isolation is costly and stressful to patients [15], and because polymerase chain reaction (PCR) for MTBC is more sensitive than smear microscopy [16–18], discontinuing isolation on the basis of a single negative sputum PCR results would allow a shorter isolation time for those who do not have (contagious) tuberculosis, as long as such an approach does not compromise culturing for MTBC, which is crucial for diagnosing paucibacillary tuberculosis cases as well as for drug susceptibility testing, differential diagnoses, and surveillance (purposes) [19, 20].

Previously, in 493 hospitalized patients with suspected tuberculosis, Campos et al [24] found that all 35 smear-positive tuberculosis cases were identified with a first-sample PCR. More recently, a few studies reported that GeneXpert MTBC/RIF (Cepheid) was just as sensitive as serial smears [21, 22]. These prospective studies were limited by the low number of initially included tuberculosis cases. This implies a risk of overlooking a low frequency of smear-positive, PCR-negative patients, which would imply a risk of in-hospital transmission. To overcome this problem, we performed a retrospective, nationwide evaluation of the feasibility and safety of relying on the first available PCR result for ruling out contagious tuberculosis.

**METHODS**

In Denmark, culture-based diagnostics are centralized at the International Reference Laboratory of Mycobacteriology at Statens Serum Institut (SSI), Copenhagen, Denmark. This allowed us to study nationwide data on culture-confirmed tuberculosis. We analyzed all data on mycobacteria provided by smear microscopy samples, PCR, and culture from 1 January 2002 to 31 December 2011. Samples from overseas (mainly Greenland) were excluded to avoid any influence of long transportation time. We included patients with ≥1 sample that was culture positive for MTBC (excluding *Mycobacterium bovis* subsp *BCG*).

We started by identifying patients with ≥3 samples within 14 days before and after the first culture-positive sample and with ≥1 PCR result. From this population, we selected the first 3 samples, prioritizing the first culture-positive sample and the first PCR result. Subsequently, we repeated the selection process and included all patients with ≥2 samples within 14 days before or after the first culture-positive sample and ≥1 PCR result. Smear positivity was defined by acid-fast bacilli (AFB) seen with auramine-rhodamine fluorescence microscopy in ≥1 of the included samples. PCR positivity was defined by identification of MTBC DNA in the first available sample for which PCR was performed. Any subsequent PCR results were excluded.

All specimens were processed by conventional procedures for unsterile specimens. Smears were stained with auramine-rhodamine fluorochrome (SSI Diagnostika) and examined with fluorescence microscopy at ×200 magnification. The AFB content in the whole slide was assessed semiquantitatively, with smear grade reported on a scale from 0 to 3 (where 0 indicates no AFB and 3, numerous AFB). After pretreatment, 500 µL of each specimen was inoculated in BACTEC Mycobacteria Growth Indicator Tube 960 liquid medium (Becton Dickinson), and 150 µL was inoculated onto Lowenstein-Jensen slants (SSI Diagnostica). Lowenstein-Jensen slants were examined weekly for growth, and the BACTEC system provided continuous monitoring. Presumed mycobacterial growth was confirmed by Ziehl-Neelsen staining. If no growth was observed after 8 weeks of incubation, the specimen was reported as culture negative. PCR for MTBC was performed using the BDProbeTec ET Direct Detection (Becton Dickinson) and GenoType MTBDRplus (Hain Life-science) assays. The International Reference Laboratory of Mycobacteriology process approximately 23 000 samples annually. All diagnostic tests performed in the laboratory are included in an external quality assurance program with good scores, and the laboratory has International Organization for Standardization 17025 accreditation [23]. Isolates from new culture-positive patients are genotyped routinely (MIRU-VNTR, available at: www.miru-vntrplus.org), as well as samples culture positive ≥3 months after the first positive sample [24, 25]. Genotyping is used for epidemiological surveillance but also to systematically control for possible cross-contamination. Any potentially contaminated samples were investigated.

The PCR assays described above are routinely used in combination with other PCR-/DNA-based methods at SSI. In Denmark, GeneXpert MTB/RIF has not been implemented for standard use; other commercial PCR/DNA methods are preferred in tuberculosis diagnostics owing to higher costs associated with GeneXpert, higher volumes of specimens required (leaving less volume for other analyses), and nearly no rifampicin resistance in the country (on average, 2 cases per year). In addition to test results, the data included some information on sample quantity (eg, if sample volume was <2 mL). Clinical information was limited to notes from the requester and, because optional, was most often incomplete.
Table 1. Patient Age and Sex, Smear Status, and Occurrence of Low-Volume Samples

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>MTBC Culture Negative</th>
<th>MTBC Culture Positive</th>
<th>PCR on ≥1 Initial Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>P Value</td>
<td>No PCR</td>
<td>Total</td>
</tr>
<tr>
<td>Patients, No.</td>
<td>20,928</td>
<td>. . .</td>
<td>699</td>
<td>937</td>
</tr>
<tr>
<td>Age, median (IQR), y</td>
<td>55 (40–68)</td>
<td>56 (41–69)</td>
<td>42 (31–52)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Sex, No.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Male</td>
<td>12,261</td>
<td>.</td>
<td>464</td>
<td>593</td>
</tr>
<tr>
<td>Female</td>
<td>8,667</td>
<td>.</td>
<td>235</td>
<td>342</td>
</tr>
<tr>
<td>Smear statusc</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Negative</td>
<td>478</td>
<td>. . .</td>
<td>224</td>
<td>254</td>
</tr>
<tr>
<td>Positive</td>
<td>1,158</td>
<td>475</td>
<td>683</td>
<td>671</td>
</tr>
<tr>
<td>Smear grade, mean (95% CI)</td>
<td>. . .</td>
<td>. . .</td>
<td>2.21 (2.15–2.26)</td>
<td>. . .</td>
</tr>
<tr>
<td>≥1 low-volume sample (&lt;2 mL), No. (%)</td>
<td>4,350 (20.8)</td>
<td>4,050 (21.0)</td>
<td>295 (18.0)</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; IQR, interquartile range; MTBC, Mycobacterium tuberculosis complex; PCR, polymerase chain reaction.

* Data on initial samples only (up to 14 days before and after the first MTBC culture positive sample); up to 3 initial samples are included.
* Significantly different sex and smear status distribution than in patients with definite PCR result.
* Smear status was considered positive if acid-fast bacilli (AFB) were seen with auramine-rhodamine fluorescence microscopy in ≥1 initial sample.
* Smear grade was assessed semiquantitatively at ×200 magnification, on a scale of 1 (no AFB) to 3 (numerous AFB).
Statistical Analysis and Ethical Considerations
The study was approved by the Danish Data Protection Agency. The study was registry based, and participants were anonymous; individual consent and ethical approval were thus not required according to Danish legislation. Data processing was performed using Stata software (version 13; StataCorp). We compared medians, means, and proportions using Mann–Whitney U, Student t, and Pearson χ² tests. For all tests, differences were considered significant at .05.

RESULTS
Population Characteristics
We identified 74,790 sputum samples within the study period; 53,533 of these were from Denmark, the rest mainly from Greenland. The samples represented 20,928 patients, 12,261 (58.6%) men and 8,667 (41.4%) women (Table 1). The overall median age at first sample was 55 years, and the median age at first culture-positive sample was 42 years (P < .01 for difference; Table 1) (Figure 1). The mean number of cultured samples per person in the study period was 2.56. Of the 20,928 patients, 9,084 (44.4%) had ≥3 samples cultured during the study period, 3,832 (18.3%) had 2 samples cultured, and 8,012 (38.3%) had only 1 sample cultured. Within the study period, 4,206 sputum samples from 1,636 patients were culture positive for MTBC (Figure 2). Of these, M. tuberculosis grew in 4,200 samples, Mycobacterium africanum grew in 3 samples from 1 patient, and M. bovis grew in 3 samples from 2 patients; 3,015 (71.7%) of the culture-positive samples were smear positive.

Results From Patients With ≥3 Initial Samples
A total of 1,274 culture-positive patients had ≥3 sputum samples evaluated in the study period, of whom 856 (52% of culture-confirmed tuberculosis patients) had ≥3 samples evaluated within 14 days before or after the first culture-positive sample. Of these, 486 had PCR for MTBC performed on ≥1 initial sample (336 had PCR performed on the first included sample, 150 on a subsequent sample); 482 had a definite smear and PCR status (no inconclusive results). Of these, 357 (74.1%) were smear positive, and 394 (81.4%) were PCR positive (Table 2). Nine patients (2.5% of the smear-positive population) were smear positive but PCR negative (Table 2).

Results From Patients With ≥2 Initial Samples
A total of 1,466 culture-positive patients had ≥2 samples evaluated in the study period, whereof 1,234 patients (75.4% of culture confirmed cases) had ≥2 samples evaluated within 14 days before and after the initial culture-positive sample. Of these, 733 had PCR for MTBC performed on ≥1 initial sample (529 had PCR performed on the first included sample, 204 on the
second); 722 of these had a definite smear and PCR status (no inconclusive results). Of these, 528 (73.1%) were smear positive, and 607 (84.1%) were PCR positive (Table 2). Seven patients (1.3% of smear-positive patients in this group) were smear positive but PCR negative (Table 2). Four patients were represented in both the 3-sample and the 2-sample analyses; thus, our study revealed 12 unique patients with culture-confirmed tuberculosis with serial smear-positive and PCR-negative results (Table 3).

**Characteristics of Smear-Positive, PCR-Negative Patients**

We included samples received at the laboratory up to 14 days before or after the first culture-positive sample (up to 28 days apart). Most samples were received within a few days; for the 3-sample groups, the median duration from first to third sample was 1 day (interquartile range [IQR], 0–3 days). For the 2-sample population, the median duration between samples was 0 days (IQR, 0–2 days) (Table 3). We found a trend toward longer intervals from first to last sample for patients with smear-positive, PCR-negative results, though this difference was insignificant in the 3-sample (4 days; IQR, 1–6 days; P = .08) and 2-sample (2 days; IQR, 0–5; P = .13) populations. The 12 smear-positive, PCR-negative patients did not differ from the overall culture-positive population with regard to age (median, 36.5 vs 42 years; P = .42) Smear positive samples from PCR-negative/smear-positive patients had significantly lower smear grades than those patients without discordant results (mean smear grade, 0.7 vs 2.21; P < .001).

**PCR in Culture-Negative Patients**

From the total population of 53,533 patients, 65 had ≥1 PCR-positive sample despite no MTBC-positive cultures. Of these, nontuberculous mycobacteria (NTM) grew in ≥1 sample in 3 (Mycobacterium avium in 1 and Mycobacterium gordonae in 2), M. bovis, subsp. BCG, grew in 1, and 9...
had ≥1 inconclusive sample. It is likely that they also received treatment before sampling, resulting in the negative cultures (no live bacteria).

**Sample Volume and the Impact on Test Results**

Of the 53,533 samples included, 6,749 (12.6%) were of low volume (<2 mL). Samples of low volume were less often MTBC culture positive (6.64%) than samples of sufficient volume (8.35%) (odds ratio [OR], 0.81; \( P < .001 \)). Culture-positive samples of low volume were less likely to be smear positive (266 of 448) than those of sufficient volume (2,749 of 3,758) (OR, 0.54; \( P < .001 \)). We also observed an insignificant trend of lower frequency of PCR positivity in low-volume samples (low volume, 140 positive of 164; sufficient volume, 1,294 positive of 1,461; OR, 0.75; \( P = .25 \)).

**DISCUSSION**

This study confirms prior findings that PCR testing of an initial sputum sample identifies the vast majority of patients with AFB in ≥1 of 2 or 3 initial sputum smears. Thus, PCR can potentially supplement isolation and shorten isolation time when the result is negative. Notably, the few smear-positive patients that were not identified by PCR nearly all had low-grade smears. PCR-negative/smear-positive patients in the analysis on the same sample did not occur in our analysis. Prior evaluations found that PCR identified all patients with tuberculosis with AFB in serial smears [22, 26], although 1 study evaluating a 1-, 2-, and 3-sample PCR (GeneXpert MTBC/RIF) approach missed 1 of 6 smear-positive tuberculosis cases with the 1-sample approach [21]. Although a PCR-based algorithm for isolation

<table>
<thead>
<tr>
<th>Initial Samples</th>
<th>Negative PCR Results</th>
<th>Positive PCR Results</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 initial samples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smear negative</td>
<td>79</td>
<td>46</td>
<td>125</td>
</tr>
<tr>
<td>Smear positive</td>
<td>9</td>
<td>348</td>
<td>357</td>
</tr>
<tr>
<td>Total</td>
<td>88</td>
<td>394</td>
<td>482</td>
</tr>
</tbody>
</table>

Abbreviation: PCR, polymerase chain reaction.

a Eleven patients with inconclusive smear status were excluded. Smear status was considered positive if auramine-rhodamine smear microscopy revealed acid-fast bacilli in ≥1 of the included samples. PCR status refers to the first available PCR results; subsequent PCR results were excluded.
decision is relatively safe, our data demonstrated that the cost of the shorter general isolation time means a potentially elevated risk of in-hospital transmission. This may have particular implications in settings with many immunosuppressed patients. However, the patients released from isolation based on a negative PCR result would most likely be isolated again based on any subsequent positive smear(s), reducing the possible transmission time.

Although Centers for Disease Control and Prevention guidelines state that the 3 sputum samples necessary for reliable smear microscopy can be collected over 2 days [5], the actual process often takes up to 5 days [26]. A conventional PCR result will most often be available within 2 days; thus, the benefit of allowing discontinuation of isolation on the basis of single-sputum PCR results is a reduction of the time spent in isolation for patients who do not have contagious tuberculosis. Previously, PCR-based isolation decision models with fast-access PCR (Xpert RIF/MTB) were found cost-effective [21, 27, 28]. One study using a conventional PCR technique (Cobas Amplicor; Roche Diagnostics) [29] did not find this cost-effectiveness, mainly because PCR was performed only twice weekly and on all samples. With access to daily PCR analysis, and with prioritization of an initial PCR sample in addition to culture, conventional PCR would probably be just as cost-effective.

In none of the 12 smear-positive, PCR-negative cases did NTM grow in addition to MTBC, which could have explained these positive smears [30]. Patients with NTM are regularly found among those with suspected tuberculosis [31], possibly resulting in unnecessary isolation in the case of positive smears. However, PCR-positive/culture-negative results can occur for multiple reasons and occurred in 65 cases in the current study. NTM grew in some of these, which may hide the presence of MTBC. Other results were inconclusive owing to growth of other bacilli, which might also hide MTBC. Importantly, of 29 patients with PCR-positive, MTBC culture-negative samples for whom clinical information was provided, 21 either had had tuberculosis previously or were currently being treated for it and were possibly presenting dead mycobacteria and thus culture negative. This emphasizes the importance of clinical judgment in interpreting PCR results and the need to secure specimens for culturing before initiating treatment.

Approximately 10%–20% of patients with tuberculosis are infected by a smear-negative source [32–35]; thus, isolating all PCR-positive patients, regardless of smear status, would probably reduce the small though not negligible, risk of in-hospital transmission from smear-negative patients. Although our study design did not allow us to draw evidence-based conclusions on cost-effectiveness of isolation strategies, reduced time in isolation for those who turn out not to have contagious tuberculosis (or tuberculosis at all) would probably reduce overall costs, despite isolation of some smear-negative patients.

Our data also outline an important pitfall in diagnosing pulmonary tuberculosis: the quality and quantity of samples are often suboptimal or variable, reducing the sensitivity of culture-based and molecular methods. Among 12 PCR-negative and serial smear-positive patients, none had both PCR-negative and smear-positive results in the same sample. The discordant results therefore probably originated from samples of varying quality (ie, too little volume or not residing from the lower respiratory tract). Notably, 5 of the 12 patients also had a positive PCR result with a subsequent sample within 28 days. Methods for improving sample quality include sputum induction [36, 37], although instruction by a healthcare professional seems equally effective and comparatively cheaper [38]. In the current study, we found that low-volume (<2-mL) samples were less often culture positive, and those that were culture-positive were less often smear positive. However, we cannot conclude whether this issue is quantitative (lower total number of bacilli in a smaller volume) or patient related (a higher concentration of lung bacilli might lead to higher sputum production). An effort to reduce the frequency of both low-quality and low-quantity samples will very likely limit the time to diagnosis and reduce costs for additional diagnostic procedures.

Although initial PCR analysis can be used to reduce time in isolation when results are negative, serial samples for culturing are crucial for adequate diagnostic sensitivity, in particular for finding the diagnostically challenging smear- and PCR-negative patients. Therefore, any specimen examined with PCR should also undergo culturing, which remains the reference standard for diagnosing tuberculosis in Europe [19]. This is also important for detecting NTM, which is becoming increasingly important in clinical practice [30, 39, 40]. Furthermore, mycobacterial culturing is crucial for drug sensitivity testing [20], genotyping, and outbreak surveillance.

Our study has some limitations. First, our data came from a single quality assured international reference laboratory, highly specialized in mycobacteriology. Thus, the PCR and smear results might not be directly attributable to the wide range of clinical settings where PCR and smears are performed by local laboratories. Second, we included only sputum samples, excluding, for example, gastric and bronchoalveolar lavage samples. In daily clinical practice, many patients are examined using different specimen materials (eg, bronchoalveolar or gastric lavage samples in addition to a single sputum sample). Third, we do not know the number of patients isolated in the hospital for suspected tuberculosis, because we studied only culture-confirmed cases retrospectively. In reality, many patients may have been evaluated as outpatients. Therefore, we cannot determine the actual number of reduced
isolation days or the threshold for isolating patients with suspected tuberculosis by relying on PCR results. Finally, PCR was performed in only a proportion of the samples when requested by clinicians, and, owing to the retrospective design, we had no knowledge of the reasons for requesting or not requesting PCR. Clinicians may have selectively requested PCR based on the clinical presentation, which may have biased our results.

In conclusion, a single primary conventional sputum PCR test identified >97% of serial smear-positive pulmonary tuberculosis cases. Most missed cases had low-grade smears. The occurrence of smear-positive, PCR-negative cases underlines the importance of increasing both the quantity and quality of the samples investigated. Still, it is important to culture samples analyzed with PCR owing to the higher sensitivity of the samples investigated. Nevertheless, it is important to perform drug susceptibility testing, differential diagnosis, and surveillance. The challenge can be overcome by using the same sample for both PCR and culturing.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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