Polymerase Chain Reaction for *Mycobacterium tuberculosis*<sup>*</sup>

Impact on Clinical Management of Refugees With Pulmonary Infiltrates

Gerd Laifer, MD; Andreas F. Widmer, MD, MS; Reno Frei, MD; Werner Zimmerli, MD; and Ursula Fluckiger, MD

**Study objectives:** Screening for pulmonary tuberculosis (TB) in war refugees entering low-prevalence countries for TB is a common policy, but workup strategies are difficult and expensive.

**Design:** Prospective screening of war refugees for TB by chest radiograph and evaluation of the impact of additional polymerase chain reaction (PCR) testing for *Mycobacterium tuberculosis* complex (MTB) on clinical management in case of pulmonary infiltrates suspicious for TB.

**Setting:** Academic university medical center.

**Patients:** A total of 3,119 adult war refugees from the Kosovo war were screened by chest radiograph on arrival. Refugees with pulmonary infiltrates suspicious for TB were hospitalized, and a standardized diagnostic workup was performed.

**Measurements and results:** Of 3,119 adult war refugees screened for TB, 29 patients (0.9%) were identified with pulmonary infiltrates suspicious for TB; 103 specimens (76 sputa; 27 BAL fluids) were collected for acid-fast smear (AFS), PCR, and culture. The prevalence of culture-proven TB infection in this population was 27.6%. Sensitivity for PCR was higher compared with AFS for all specimens (64% vs 20%; \( p < 0.01 \)) and also for each refugee with at least one positive specimen finding (100% vs 37.5%; \( p = 0.025 \)). More important, the negative predictive value for three consecutive PCRs (in two sputa and one BAL) was 100%.

**Conclusions:** Repeated PCR testing for MTB in a population of asymptomatic war refugees with pulmonary infiltrates highly suggestive of TB is significantly more sensitive than AFS. Three negative PCR results allow discharge from isolation, thus reducing the economic burden of isolation strategies.

**Key words:** acid-fast smear; asymptomatic; *Mycobacterium tuberculosis*; polymerase chain reaction; pulmonary tuberculosis; refugees

**Abbreviations:** AFS = acid-fast smear; MTB = *Mycobacterium tuberculosis* complex; NPV = negative predictive value; PCR = polymerase chain reaction; PPV = positive predictive value; TB = pulmonary tuberculosis

---

The worldwide pulmonary tuberculosis (TB) epidemic, with emerging drug resistance and a growing number of refugees from different parts of the world, has increased the clinical impact of tuberculosis control in refugee populations. TB screening of all immigrants to low-prevalence countries is justified by high incidence rates of TB in this population in several studies from Europe and the United States.\(^1\)\(^-\)\(^3\) These studies also provide evidence that TB can be controlled with a limited screening program of chest radiographs on arrival.\(^1\)\(^,\)\(^2\)

In Switzerland, the TB incidence rate of refugees is approximately 45 times higher than that of the indigenous population (273/100,000 vs 5.9/100,000).\(^4\) Screening for TB by chest radiography is mandatory for all refugees on arrival, followed by clinical evaluation in case of a pulmonary infiltrate. However, the diagnosis of active TB is difficult because most refugees are asymptomatic with a low mycobacterial load, associated with a low sensitivity of sputum tests for acid-fast smear (AFS).

Currently available rapid tests lack appropriate
sensitivity and specificity to provide an accurate diagnosis of early stage TB. The most widely used rapid test is direct microscopy examination of a smear of sputum for acid-fast bacilli. However, sensitivity varies widely depending on the clinical and radiologic presentation, ranging from 22 to 78%.5-7 This technique allows rapid diagnosis in patients with advanced disease9 but fails at earlier stages. It would be of considerable epidemiologic and clinical interest to be able to diagnose TB while patients are still smear negative, because at this stage they are less contagious9,10 and have a lower morbidity and mortality.11

Nucleic acid amplification techniques have a sensitivity and specificity > 95% in smear-positive specimens.12-14 The polymerase chain reaction (PCR) technique offers higher accuracy than AFS at greater speed than culture, and may thus overcome problems associated with these conventional diagnostic tools. However, in smear-negative samples the sensitivity of PCR is between 40% and 77%, while specificity remains high.15,16 In addition, the capacity of detecting small numbers of microorganisms raises the concern of cross-contamination with a significant number of false-positive results.17,18 A combination of tests may improve diagnostic accuracy, shorten the duration of hospitalization, and decrease the time of isolation in rooms with negative pressure.

The goal of our study was to investigate whether repeated PCR testing combined with direct microscopy increases the sensitivity and specificity of rapid tests in a highly selected population of war refugees with pulmonary infiltrates suspicious for TB. In addition, we tested the impact of these results on the clinical management in this setting.

**Patients and Methods**

**Study Population**

Between March and September 1999, we prospectively studied all refugees coming from the Kosovo war region to one of five refugee camps in Switzerland. Refugees with pulmonary infiltrates suspicious for TB on screening radiograph at arrival were referred to the University Hospitals Basel. They underwent a standardized protocol (Fig 1) with sputum examination and bronchoscopy with BAL in order to obtain consecutive specimens on three occasions. Hospital records were reviewed for demographic data and clinical information.

**Microbiological Processing of Clinical Samples**

All respiratory specimens were digested and decontaminated by the NaOH – N-acetyl-L-cysteine method19 and then centrifuged (15 min at 3,000g). The sediment was mixed with sterile water to a 1:10 dilution, stained with auramine O fluorochrome, and examined by fluorescence microscopy. Ziehl-Neelsen acid-fast staining was used to confirm the presence of acid-fast bacilli. Mycobacterial cultures were performed by sample inoculation onto Loewenstein-Jensen media (Bio-Rad Laboratories; Hercules, CA), Middlebrook 7H10 and selective 7H11 media (Becton-Dickinson; Franklin Lakes, NJ), as well as into liquid medium (radiometric Bactec 460 system; Beckton-Dickinson) according to instructions of the manufacturer, followed by incubation at 37°C for 8 weeks. PCR testing for *Mycobacterium tuberculosis* complex (MTB) was performed using the Roche Amplicor Mycobacterium test (Roche; Basel, Switzerland) on concentrated decontaminated specimens according to instructions of the manufacturer.

**Case Definition**

**Active TB (“Gold Standard”):** Active TB was deemed present if sputum culture or BAL findings were positive for MTB, or clinical (culture negative) diagnosis in case of compatible abnormal chest radiograph followed by both clinical and radiologic improvement after therapy for 2 to 3 months with standard combination therapy without alternative diagnosis.

**Inactive TB:** Inactive TB was deemed present if culture findings for MTB were negative in case of an abnormal chest radiograph remaining stable during standard therapy for 2 to 3 months in suspected TB cases or alternative pulmonary diagnosis found.

**Stable Radiographic Scarring:** Stable radiographic scarring was deemed present if all culture findings for MTB were negative, in addition to stable abnormal findings of chest radiograph during follow-up, and no alternative pulmonary diagnosis was found.

**Radiographic Criteria for Suspicious Active TB:** Radiographic criteria for suspicious active TB included diffuse pulmonary infiltrate not meeting radiographic findings for prior (inactive) disease (ie, apical fibronodular infiltration with volume loss; calcified solitary pulmonary nodules; calcified hilar lymph nodes; and pleural thickening.20

**Alternative Pulmonary Diagnosis:** Alternative pulmonary diagnoses included infections, cancer, and noninfectious diseases (ie, sarcoidosis) diagnosed by the physicians in charge, explaining the abnormal chest radiograph finding and the clinical picture.

**Statistical Methods**

Results of diagnostic tests were compared by applying the χ² test for categorical variables. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated according to Rosner,21 using the above-mentioned “gold standard.”

**Results**

Between March and September 1999, 4,758 war refugees from Kosovo came to Basel, Switzerland; 3,119 refugees (65.5%) were adults and were screened by chest radiograph at arrival. Twenty-nine refugees (0.9%) were referred to the hospital for evaluation of a possible TB (Table 1). All of them had pulmonary infiltrates suspicious for TB on chest radiograph. In addition, a cavity or nonspecific pleural scarring was present in 5 of 29 patients (17.2%) and 15 of 29 patients (51.7%), respectively. Twenty of 29 patients (69.0%) had pulmonary symptoms (Table 1). A total of 103 respiratory specimens were
collected (76 sputa and 27 BAL samples). Eight refugees (0.3%) had active TB diagnosed, representing a TB incidence rate of 256/100,000. The prevalence of active TB in the cohort with suspected TB was 27.6%. The calculated sensitivity, specificity, PPV, and NPV are summarized in Table 2. The

**Figure 1.** Proposed workup for refugees from high-prevalence countries with pulmonary infiltrates suspicious for TB on screening radiograph at hospital admission. pos = positive; neg = negative; NTM = nontuberculous Mycobacteria; pts = patients; DD = differential diagnosis; 3xneg = three negative AFS/PCR; 1xpos = one positive AFS/PCR; 2xpos = two positive AFS/PCR.
sensitivity of PCR testing was higher than that of AFS in overall specimens (64.0% vs 20.0%; p < 0.01) and for all refugees with at least one positive specimen (100% vs 37.5%; p = 0.025). All refugees with culture-positive active TB (n = 8) had at least one positive PCR result, and five of eight patients (62.5%) had more than one positive PCR result, whereas only three of eight patients (37.5%) had at least one positive AFS result. The specificity of AFS was 100% for overall specimens and for all patients (three specimens per patient), while there were three false-positive PCR results yielding a specificity of 96.2% for all specimens and 85.7% for all refugees. Accordingly, PPV was lower for PCR than for AFS (84.2% vs 100% for all specimens; 72.7% vs 100% for all refugees). The NPV for all refugees with three respiratory specimens was 100% for PCR vs 80.8% for AFS, suggesting that it is safe to discharge refugees from isolation after obtaining the third negative PCR result.

The results of negative rapid diagnostic tests did not influence the decision of clinicians to start or withhold empiric therapy. Antituberculous therapy was started in all patients with one or more positive PCR or AFS result (11 of 11 patients), as it was in an additional 10 of 18 refugees with negative results from rapid diagnostic tests but a high suspicious clinical picture without alternative pulmonary diagnosis. In seven refugees, an alternative diagnosis was made (Table 3). Therapy was reevaluated after obtaining culture results and clinical and radiologic follow-up consultation after 2 to 3 months. All culture-positive patients (n = 8, all HIV negative) received a 6-month course of treatment; in 10 of 13 culture-negative refugees, empirical therapy was discontinued after follow-up investigation. In 3 of 13 refugees with inactive TB, a full-course therapy for 6 months was administered because of extensive radiologic findings in previously untreated younger patients. The median duration of isolation in TB-negative patients was 9 days (range, 3 to 28 days), with a longer stay in the three patients with one false-negative PCR result (27 days or 28 days, respectively).

Molecular typing of six strains could not detect a specific strain cluster of MTB (data not shown). Lack of clustering suggests no ongoing transmission among refugees and implies that their infection was acquired independently.

**Discussion**

Diagnosing active TB is difficult in asymptomatic patients with minimal pulmonary infiltrates. No single ideal rapid test is currently available that combines high sensitivity and specificity. Clinical guidelines recommend obtaining at least three sputum specimens for AFS and culture, preferably collected on separate days, in order to confirm or to rule out TB. We were able to demonstrate that

### Table 2—Epidemiologic and Clinical Characteristics of Refugees*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All Refugees (n = 3,119)</th>
<th>Refugees With Active TB (n = 8)</th>
<th>Refugees Without Active TB (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1,728 (54.4)</td>
<td>7 (87.5)</td>
<td>12 (57.1)</td>
</tr>
<tr>
<td>Female</td>
<td>1,391 (44.6)</td>
<td>1 (12.5)</td>
<td>9 (42.9)</td>
</tr>
<tr>
<td>Age, yr</td>
<td>27 (16-91)</td>
<td>26 (21-68)</td>
<td>56 (20-79)</td>
</tr>
<tr>
<td>Chest radiograph findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infiltrates</td>
<td>8 (100)</td>
<td>21 (100)</td>
<td></td>
</tr>
<tr>
<td>Cavity</td>
<td>5 (62.5)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Nonspecific changes</td>
<td>2 (25)</td>
<td>13 (61.9)</td>
<td></td>
</tr>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td>6 (75)</td>
<td>14 (66.7)</td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>3 (37.5)</td>
<td>4 (19)</td>
<td></td>
</tr>
<tr>
<td>Weight loss</td>
<td>2 (25)</td>
<td>4 (19)</td>
<td></td>
</tr>
<tr>
<td>Night sweats</td>
<td>5 (62.5)</td>
<td>5 (23.8)</td>
<td></td>
</tr>
<tr>
<td>Isolation, d</td>
<td>≥ 121</td>
<td>9 (3-25)</td>
<td></td>
</tr>
<tr>
<td>Hospitalization, d</td>
<td>≥ 121</td>
<td>9 (3-25)</td>
<td></td>
</tr>
<tr>
<td>Initial antitubercular therapy</td>
<td>8 (100)</td>
<td>13 (61.9)</td>
<td></td>
</tr>
<tr>
<td>6-mo regimen</td>
<td>8 (100)</td>
<td>3 (14.3)</td>
<td></td>
</tr>
<tr>
<td>Stop after reevaluation</td>
<td>0 (0)</td>
<td>10 (47.6)</td>
<td></td>
</tr>
</tbody>
</table>

*Data are presented as No. (%) or median (range).
†Most refugees referred to other clinics for completion of therapy.

### Table 2—Sensitivity, Specificity, PPV, and NPV of Rapid Diagnostic Tests for MTB in Refugees With Pulmonary Infiltrates*

<table>
<thead>
<tr>
<th>Variables</th>
<th>AFS</th>
<th>PCR</th>
<th>p Value</th>
<th>AFS</th>
<th>PCR</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>5/25  (20.0)</td>
<td>16/25 (64.0)</td>
<td>&lt; 0.01</td>
<td>3/8   (37.5)</td>
<td>8/8   (100)</td>
<td>0.025</td>
</tr>
<tr>
<td>Specificity</td>
<td>78/78 (100)</td>
<td>75/75 (96.2)</td>
<td>NS</td>
<td>21/21 (100)</td>
<td>18/21 (85.7)</td>
<td>NS</td>
</tr>
<tr>
<td>PPV</td>
<td>5/5   (100)</td>
<td>16/19 (84.2)</td>
<td>NS</td>
<td>3/3   (100)</td>
<td>8/11 (72.7)</td>
<td>NS</td>
</tr>
<tr>
<td>NPV</td>
<td>78/88 (79.6)</td>
<td>75/84 (89.3)</td>
<td>NS</td>
<td>21/26 (80.8)</td>
<td>18/18 (100)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Data are presented as No. of specimens/respective patients (%). NS = not significant.
†With one or more positive specimen.
Table 3—Diagnoses at Discharge of 29 Refugees With Pulmonary Infiltrates on Screening Chest Radiograph Suspicious for TB on Hospital Admission

<table>
<thead>
<tr>
<th>Diagnoses</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB-related</td>
<td></td>
</tr>
<tr>
<td>Culture-positive TB</td>
<td>8 (27.6)</td>
</tr>
<tr>
<td>Extended nonspecific changes but no active TB</td>
<td>14 (48.3)</td>
</tr>
<tr>
<td>Alternative diagnosis</td>
<td></td>
</tr>
<tr>
<td>Bacterial pneumonia (Streptococcus pneumoniae)</td>
<td>2 (6.9)</td>
</tr>
<tr>
<td>Bacterial bronchitis (concomitant nonspecific changes)</td>
<td>2 (6.9)</td>
</tr>
<tr>
<td>Sarcoïdosis</td>
<td>2 (6.9)</td>
</tr>
<tr>
<td>Interstitial lung disease of unknown cause</td>
<td>1 (3.4)</td>
</tr>
</tbody>
</table>

three negative PCR results for MTB from respiratory specimens (including one BAL) had a predictive value of 100% in excluding active TB in our patients, whereas the NPV of three AFS specimens alone was only 80.8%. With one single PCR test of respiratory specimens, the NPV was 89.3%. This is not sufficient for the clinical management of these patients, in particular for decisions regarding isolation, confirming the results of a similar study in a different population.25

The sensitivity of PCR testing in our study was significantly higher than that of AFS. AFS had a low diagnostic yield in our patients (20.0% in all specimens and 37.5% for all patients with three specimens), consistent with results from other studies with minimal pulmonary TB.7,8 The specificity of our PCR tests (96.2% for all specimens) was in the range of recently published data.12–16 Limitations of PCR tests are the possible presence of inhibitory substances leading to false-negative results26 and nucleic-acid cross-contamination leading to false-positive results.12 These limitations of PCR and AFS underscore the need to combine or repeat tests, especially in patients with minimal TB.

Several studies have stressed the importance of a clinical risk assessment providing important information regarding predictive values. An estimation of pretest probability is crucial for the interpretation of a rapid diagnostic test for MTB in an individual patient in order to discriminate accurately between high, intermediate, and low risk of pulmonary TB.25,27 This risk assessment is helpful in targeting areas of the clinical spectrum in which nucleic acid amplification tests can make important contributions in diagnosing TB. The American Thoracic Society has recommended that these tests should be interpreted at different levels of clinical suspicion.15 There can be no doubt of the desirability of a test with a NPV high enough that patients could be safely released from isolation without an increased risk of transmission of MTB in asylum facilities or general wards to patients who are at high risk of contracting TB. However, PPV of PCR fails to be a reliable tool for diagnosis of TB as single test, and should only be introduced as a complementary diagnostic method together with staining to increase the number of TB cases detected before culture results are available. Despite improved diagnostic tools, a substantial proportion of TB suspects will remain, in whom treatment decisions will be made solely based on clinical judgment.

TB is a major epidemiologic problem among immigrants and refugees. In one study28 in the United States, 6.9% of refugees seeking medical care had active TB and almost 40% were candidates for preventive therapy. In the United States, the TB case rate is four to five times higher in foreign-born persons than in US-born residents.29 The TB incidence rate of 256/100,000 in our study is similar to other reports in asylum seekers from Europe.1,2,4,30 In our highly selected study population of war refugees from Kosovo, 27.6% of asymptomatic persons with pulmonary infiltrates on radiographs had culture-positive active TB confirming the need for strict screening and management programs for immigrants and war refugees from high-prevalence areas when they enter low-prevalence countries.

In acute care settings, only one of 8 to 10 suspected cases turn out to have active TB,31 resulting in high costs for unnecessary respiratory isolation. We present a straightforward diagnostic policy (Fig 1) that can decrease isolation days and thus reduce costs. We recommend obtaining three respiratory specimens (preferable two morning sputa collected on separate days and one BAL) for AFS and PCR. If AFS and PCR for MTB are negative in all three samples, patients can be released from isolation after 2 to 3 days.

REFERENCES

5 Boyd JC, Marr JJ. Decreasing reliability of acid-fast smear techniques for detection of tuberculosis. Ann Intern Med 1975; 82:489–492
6 Strumpf IJ, Tsang AY, Sayre JW. Reevaluation of sputum


