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Peripheral-blood-based PCR assay to identify patients with active pulmonary tuberculosis

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Summary

Background There is a need for rapid diagnosis of pulmonary tuberculosis. We have previously used a PCR to detect circulating *Mycobacterium tuberculosis* DNA in blood samples from patients (mostly HIV-infected) with pulmonary tuberculosis. We have now prospectively investigated the role of this blood-based PCR assay for diagnosis of this disease in a clinical setting.

Methods Our PCR assay is specific for the IS6110 insertion element of the *M tuberculosis* complex of organisms. We used it to test peripheral blood from 88 consecutive patients admitted to a chest ward with suspected pulmonary tuberculosis. Personnel who carried out the assay did not know the results of any clinical investigations and ultimate diagnosis, and clinicians did not know the PCR results. Results of the PCR assay were compared with the final clinical diagnosis. A subgroup of 15 patients had blood samples assayed serially to track the PCR signal over time.

Findings 41 patients had a final clinical diagnosis of tuberculosis, and the cases were typical of those seen at our hospital: HIV infection was common, and most cases were not sputum-smear positive for acid-fast bacilli. The PCR assay correctly identified 39 of 41 patients with proven pulmonary tuberculosis, 26 (63%) of whom were sputum-smear negative. There were five patients in whom a positive PCR result did not accord with the final clinical diagnosis, and two of the 44 negative PCR results were classified as false negatives. The overall sensitivity and specificity of the PCR assay for a diagnosis of tuberculosis was 95% and 89%, respectively. In 15 patients with pulmonary tuberculosis and a positive blood assay, the PCR result remained positive after 1 month of therapy, but had reverted to negative in 13 of the 15 by 4 months of therapy.

Interpretation We conclude that peripheral-blood-based PCR detection for the diagnosis of tuberculosis is a technically feasible approach that has a potentially important role in the diagnosis of pulmonary tuberculosis.

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Introduction

Diagnostic approaches to pulmonary tuberculosis ha been virtually unchanged for many years; sputum sme examination, sputum culture, and chest radiograp remain the most frequently used methods.[1] Howev there is an urgent need for rapid and accurate diagnosis tuberculosis. HIV infection, institutional outbrea transmission of tuberculosis, and the emergence multidrug-resistant disease underscore the urgency early identification and treatment.

Recently, developments in molecular biology ha raised hopes about the possibilities of new strategies tuberculosis diagnosis.[2,3] Most of these methods ha focused on the application of PCR to sputum samp from patients with suspected mycobacterial disea Although these approaches have been promising, t exact clinical role of the PCR assay as it applies to sputu analysis is unclear. The infectious nature of sputu (when handled in the laboratory and when collect through induction by nebulised saline), potential preser of PCR inhibitors in samples, and difficulty in obtaini samples from some patients have led us to search for m accessible biological specimens that might be use adjuncts in the diagnosis of pulmonary tuberculo Though rapid tests may not replace the need for sputu cultures generally (to look for other organisms or susceptibility testing of mycobacterial isolates), a ra test with another type of sample may reduce the numb of sputum samples that need to be collected, and negative result may allow the diagnosis of tuberculosis be ruled out quickly and with certainty.

We have previously reported in a preliminary study eight patients (mostly HIV infected) with act pulmonary tuberculosis that DNA from *Mycobacteri tuberculosis* could be amplified by PCR from periphe blood mononuclear cells.[4] We now report the results prospective evaluation of the usefulness of a PCR-ba peripheral blood assay for the diagnosis of pulmon tuberculosis.

Methods

Clinical samples

Samples of blood were taken from consecutive patients admi during a 6-month period to the Bellevue Chest Service who u undergoing diagnostic evaluation for suspected tuberculosis. blood samples were obtained in the course of routine diagno evaluation and were assayed without knowledge of the suspec diagnosis or the results of any other laboratory investigations. conventional diagnosis at least three sputum samples (range th to 12) were obtained and cultured. Blood samples from pati were coded by number after collection and delivered to laboratory with only that number as an identifier. No clin information was available to the laboratory workers doing PCR assay.

After initial diagnoses were estal ished, 15 patients culture-proven pulmonary tuberculosis had follow-up bl

	Number (%)*
Racial/ethnic distribution	
Black	44 (50)
Hispanic	19 (22)
White	15 (17)
Asian	10 (11)
HIV serostatus	
Positive	37 (42)
Negative	41 (47)
Unknown	10 (11)
Sex (M/F)	84/4
Age	
Mean	37·5 years
Range (number in range)	
0–20 years	0
21–30	11
31–40	40
41–50	21
51–60	13
61–70	3

*Unless otherwise stated.

Table 1: **Demographic characteristics of patients admitted to the chest service with suspected tuberculosis**

samples taken after 1 month and 4 months of conventional antituberculous therapy. These 15 patients were selected for further studies because they received continuing care in our clinic, whereas the other patients received follow-up care closer to their place of residence.

2 mL of whole blood from each patient was collected in tubes containing EDTA and diluted with 2 mL phosphate buffered saline (PBS). The diluted blood was carefully layered over Ficoll-Hypaque (Pharmacia, Inc) in a ratio of 3 to 4 (blood:Ficoll) and the buffy coat layer of cells was obtained with density centrifugation (700 $g \times 40$ min, brake off). The buffy coat was aspirated and then washed with PBS (700 $g \times 10$ min, brake on) in a total volume of 10 mL. Cells were suspended in 40 μL of tris-EDTA (TE) buffer (pH 7·4), and 60 μL of 10% sodium dodecylsulphate and 10 μL of freshly prepared proteinase K (20 mg/mL) were added. The cells were incubated at 56°C for 1 hour, followed by 10 min at 95°C. Phenol/chloroform extraction of DNA followed by ethanol precipitation was then done. After DNA precipitation, pellets were washed in TE and the DNA was resuspended in 20 μL of sterile double-distilled water for use in the PCR reaction.

PCR

Primers homologous to the mycobacterial insertion sequence IS6110 were used. This insertion element is specific for the *M tuberculosis* complex of organisms (including *M bovis*, *M africanum*, and *M microti*) and will not detect organisms of the *M avium* complex.[5] Nested primer pairs were used in the PCR protocol for sensitive amplification. The outer primer, designed for complementarity with the inverse terminal repeat region of the IS6110 insertion sequence consisted of oligonucleotide 5' TGAACCGCCCCGGCATGTCCGGAGACT 3'. The inner primer pair consisted of the sense primer 5' CGTGAGGGCATCGAGGTGGC 3' and the antisense primer 5' GCGTAGGCGTCGGTGACAAA 3'.

In both the initial and nested reactions, negative controls were included in each experiment. The negative control reactions consisted of buffer, nucleotides, primers, magnesium, and *Taq* polymerase, but no template. Positive controls with genomic DNA isolated from a clinical isolate of *M tuberculosis* as template were also included in each experiment. The reaction tube containing the positive control was prepared under a hood in a separate room in the laboratory to avoid contamination and product carryover, and all PCR reactions were carried out in duplicate to reduce the risk of false-positive results. PCR products were then analysed by agarose gel (2%) electrophoresis. All PCR results were interpreted by two observers, and all samples were processed twice.

	Number (%)
Sputum AFB smear result	
Positive	15 (37)
Negative	26 (33)
Chest radiographic pattern	
Parenchymal infiltrate	34 (85)
Mediastinal lymphadenopathy	3 (5)
Miliary pattern	2 (5)
Normal	2 (5)
HIV status	
Positive	18 (43)
Negative	16 (40)
Unknown	7 (17)

AFB=acid-fast bacilli.

Table 2: **Clinical features of cases of active pulmonary tuberculosis**

Results

PCR in initial diagnosis of pulmonary tuberculosis

One blood sample from each of 88 patients was obtained for PCR. Relevant demographic characteristics are shown in table 1. Four patients had received BCG vaccination previously, and 12 (14%) had a history of previous tuberculosis disease. 44 patients were PCR positive and 44 were PCR negative. 39 of 41 patients with pulmonary tuberculosis were PCR positive and 42 of 47 patients with non-tuberculous disease were PCR negative, giving a sensitivity of the PCR assay on peripheral blood of 95% and a specificity of 89%. The positive and negative predictive values were 89% and 95%, respectively.

Among the 44 PCR-positive patients, 38 had evidence of active tuberculosis as shown by a positive sputum culture; one additional patient had a clinical diagnosis of tuberculosis (without culture confirmation) and he improved both clinically and radiographically while on antituberculous therapy. The relevant clinical characteristics of this group of patients are shown in table 2; notably, apart from the two patients with a miliary pattern seen on chest radiograph, no patient had evidence of extrapulmonary involvement. Five of the PCR-positive patients had no definite clinical evidence of tuberculosis: one of them had adenocarcinoma of the lung, and of the remaining four, three were HIV seropositive, with a mean CD4 count of 293/μL (range 89–400/μL). Two of the four patients (one HIV positive) had received BCG vaccination previously, and one of the four had a diagnosis of tuberculosis made within the previous 12 months and had received antituberculous therapy before admission to our hospital.

Of the 44 PCR-negative patients, two had active tuberculosis, as judged by clinical status and sputum culture. Diagnoses in the remaining 42 were carcinoma of the lung (one), invasive aspergillosis (two), bronchitis/bronchiectasis (21), *Pneumocystis carinii* pneumonia (ten), tuberculosis infection without disease (five), and unknown (three).

Effect of therapy on PCR detection of mycobacterial DNA

15 patients with culture-proven tuberculosis and positive blood PCR results at the time of diagnosis were followed while on antituberculous therapy. After 1 month of therapy, all 15 patients remained PCR positive. At 4 months of therapy, 13 had a negative PCR result. All 15 patients had been responding to therapy clinically, and sputum cultures had become negative. Two patients remained PCR positive for *M tuberculosis*. In one of these

two patients antituberculous therapy had been interrupted because of abdominal pain; the other patient had a miliary pattern on his chest radiograph at the time of diagnosis.

Discussion

We have shown that the detection of circulating mycobacterial DNA by PCR is sensitive and specific in the diagnosis of active pulmonary tuberculosis, the results being available within a day. In our cohort, PCR of blood samples in patients with suspected tuberculosis yielded a sensitivity of 95% and a specificity of 89%. These values compare favourably to results for diagnosis of tuberculosis generally obtained by conventional methods such as sputum microscopy, and they are similar to results obtained with PCR analysis of sputum samples. The yield of sputum microscopy for the immediate diagnosis of tuberculosis has usually been reported in the 50–75% range, with smears less often positive in non-cavitary disease or in patients with HIV infection.[7,8]

In our series, positive PCR results were not affected either by the radiographic presentation or by the HIV status of the patients. In addition, recent work suggests that the positive predictive value of sputum smears for acid-fast bacilli may be higher in patients from whom respiratory samples are collected by sputum induction or bronchoalveolar lavage, since these techniques are more likely to raise samples containing organisms from the *M avium* complex.[9] Our PCR assay is specific for organisms of the *M tuberculosis* complex. Although the clinical usefulness of sputum-based PCR remains unclear, a recent well-done clinically oriented study using a commercially developed PCR kit indicates a rough equivalence between PCR and culture results, with a clinical case definition as the gold standard for diagnosis.[10] Our blood-based assay reported here has yielded similar results.

There were five patients who were PCR positive for *M tuberculosis* DNA without culture or clinical confirmation of tuberculosis. Further analysis of these patients showed that one had been given a diagnosis of tuberculosis in the months before admission to our hospital, and had received some antituberculous therapy. Two other patients had received BCG vaccination, including one with HIV infection. PCR assays based on detection of the insertion element IS6110 cannot distinguish between *M tuberculosis* and *M bovis*, and it is possible, especially in HIV-infected patients in whom clinical infection with *M bovis* may occur several years after vaccination, that this was responsible for the positive PCR result.[11] There was no obvious explanation for the other two patients with positive PCR results but without clinical or culture evidence of tuberculosis. For the calculation of sensitivity and specificity of our assay, however, we included all five cases as false-positive results.

Two patients with negative PCR results had positive sputum cultures for *M tuberculosis*, and we counted them as false negatives. Although we had speculated that these false-negative results were due to low levels of infection or early stages of disease, both patients had cavitary disease, and one was sputum-smear positive. It is possible that these two patients were infected with strains of *M tuberculosis* that did not carry the insertion sequence IS6110. Such strains are uncommon, but do exist.[12] not available for the isolates from these two false-negative patients to assess whether the insertion element was present.

Although sputum-based PCR detection of *M tuberculosis* has a similar sensitivity and specificity to those of our blood-based PCR, the procurement and processing of sputum samples for PCR reactions are often cumbersome and associated with infectious risks to the personnel collecting the specimens and doing the assay. This problem has led many investigators to search for less complex methods of sample preparation.[13] Sputum induction, an increasingly used method of sample collection, requires careful attention to infection control procedures as well as the time and effort of trained personnel.[14] A blood-based assay potentially avoids these difficulties. Additionally, all patients can provide blood samples for analysis, which is not always possible with sputum.

Sputum-based PCR assays for the diagnosis of tuberculosis remain positive for many months after antituberculous therapy has started.[15] We attempted to address this issue in our assay by obtaining serial blood samples from a subset of patients in whom we documented active pulmonary tuberculosis with a positive peripheral blood PCR signal. In all of these cases, the PCR assay remained positive after 1 month of therapy, but in 13 of 15 patients the signal had reverted to negative after 4 months of therapy. It is noteworthy, that one of the patients with a persistently positive result had interrupted his therapy, and the other patient initially presented with a miliary pattern on chest radiograph, suggesting perhaps a higher burden of bloodborne organisms.

Several caveats should be mentioned when generalising the results of this study to other populations of patients. We feel that the patients with proven tuberculosis in our study are typical of those seen in many urban hospitals in the USA: HIV infection was common, the radiographic presentations were typical, and the patients were not overwhelmingly sputum-smear positive. We do not believe that our results reflect an excess of extrapulmonary disease or an over-representation of patients with enormous bacterial burdens. Positive blood cultures for *M tuberculosis* have been reported as positive in a relatively small number of cases, usually in HIV-infected patients with advanced AIDS or extrapulmonary tuberculosis,[16,17] but in our series only two of 56 patients tested had mycobacteraemia as detected by blood cultures.

We emphasise that in our series the blood-based PCR assay was used as a diagnostic adjunct in a cohort of patients admitted to the chest ward with a high suspicion of tuberculosis. In fact, the prevalence of active tuberculosis in our cohort was 46%. Suspected cases of tuberculosis included only patients whose clinical and radiographic presentations were strongly suggestive of tuberculosis, rather than patients involved in a contact investigation, for example. In that sense, our cohort was similar to that described by Gordin and colleagues,[18] who found that 48% of smear-negative patients with strongly suggestive clinical and radiographic presentations had active disease, in contrast to a series from Kenya in which disease was suspected in patients with a cough persisting for 4 weeks or more.[19] With this less stringent definition of suspected tuberculosis, the prevalence of disease was only 4·8%. We did not attempt to use the assay as a screening modality for active tuberculosis in a population with a

used as such, we would expect the positive predictive value to decrease substantially.

Our peripheral-blood-based PCR assay for the diagnosis of tuberculosis is a rapid, sensitive, and specific test that could prove useful in certain clinical situations. The problem of product carry-over contamination may become a serious issue if DNA amplification techniques become widely used by less experienced laboratory workers. We had a laboratory environment and used an experimental protocol designed to reduce to a minimum any contamination problems. In general use, carry-over contamination can be avoided with various techniques, including the use of uracil DNA glycosylase.[20]

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References

1 Schluger NW, Rom WN. Current approaches to the diagnosis of active pulmonary tuberculosis. *Am J Respir Crit Care Med* 1994; **149:** 264–67.

2 Schluger NW, Rom WN. The polymerase chain reaction in the diagnosis and evaluation of pulmonary infections. *Am J Respir Crit Care Med* 1995; **152:** 11–16.

3 Godfrey-Frausset P. Molecular diagnosis of tuberculosis: the need for new diagnostic tools. *Thorax* 1995; **50:** 709–11.

4 Schluger NW, Condos R, Lewis S, Rom WN. DNA amplification of *Mycobacterium tuberculosis* from peripheral blood of patients with pulmonary tuberculosis. *Lancet* 1994; **344:** 232–33.

5 Hermans PWM, Schuitema ARJ, Van Soolingen D, et al. Specific detection of *Mycobacterium tuberculosis* complex strains by polymerase chain reaction. *J Clin Microbiol* 1990; **28:** 1204–13.

6 Schluger NW, Kinney D, Harkin TJ, Rom WM. Clinical utility of the polymerase chain reaction in the diagnosis of infections due to *Mycobacterium tuberculosis. Chest* 1994; **105:** 1116–21.

7 Levy H, Feldman C, Sacho H, van der Meulen H, Kallenbach J, Koornhof H. A reevaluation of sputum microscopy and culture in the diagnosis of pulmonary tuberculosis. *Chest* 1989; **95:** 1193–97.

8 Klein N, Duncanson F, Lenox T, Pitta A, Cohen S, Wormser G. Use of mycobacterial smears in the diagnosis of pulmonary tuberculosis in AIDS/ARC patients. *Chest* 1989; **95:** 1190–92.

9 Yajko DM, Nassos PS, Sanders CA, Madej JJ, Hadley WK. High predictive value of the acid-fast smear for *Mycobacterium tuberculosis* despite the high prevalence of *Mycobacterium avium* complex in respiratory specimens. *Clin Infect Dis* 1994; **19:** 334–36.

10 Chin DP, Yajko DM, Hadley WK, et al. Clinical utility of a commercial test based on the polymerase chain reaction for detecting *Mycobacterium tuberculosis* in respiratory specimens. *Am J Respir Crit Care Med* 1995; **151:** 1872–77.

11 Smith E, Thybo S, Bennedsen J. Infection with *Mycobacterium bovis* in a patient with AIDS: a late complication of BCG vaccination. *Scand J Infect Dis* 1992; **24:** 109–10.

12 Yuen LK, Ross BC, Jackson KM, Dwyer B. Characterization of *Mycobacterium tuberculosis* strains from Vietnamese patients by Southern blot hybridization. *J Clin Microbiol* 1993; **31:** 1615–18.

13 Wilson SM, Nava E, Morales A, Godfrey-Faussett P, Gillespie S, Anderson N. Simplification of the polymerase chain reaction for detection of *Mycobacterium tuberculosis* in the tropics. *Trans R Soc Trop Med Hyg* 1993; **87:** 177–80.

14 Nardell EA. Environmental control of tuberculosis. *Med Clin North Am* 1993; **77:** 1315–34.

15 Kennedy N, Gillespie SH, Saruni AOS, et al. Polymerase chain reaction for assessing treatment response in patients with pulmonary tuberculosis. *J Infect Dis* 1994; **170:** 713–16.

16 Shafer RW, Kim DS, Weiss JP, Quale JM. Extrapulmonary tuberculosis in patients with human immunodeficiency virus infection. *Medicine* 1991; **70:** 384–97.

17 Bouza E, Diaz-Lopez MD, Moreno S, Bernaldo de Quiros JC, Vicente T. *Mycobacterium tuberculosis* bacteremia in patients with and without human immunodeficiency virus infection. *Arch Intern Med* 1993; **153:** 496–500.

18 Gordin FM, Slutkin G, Schecter G, Goodman PC, Hopewell PC. Presumptive diagnosis and treatment of pulmonary tuberculosis based on radiographic findings. *Am Rev Respir Dis* 1989; **139:** 1090–93.

19 Aluoch JA, Swai OB, Edwards EA, et al. Studies of case-finding for pulmonary tuberculosis in outpatients at 4 district hospitals in Kenya. *Tubercle* 1985; **66:** 237–49.

20 Longo MC, Berninger MS, Hartley LJ. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene* 1990; **93:** 125–28.