# Polymerase chain reaction for the detection of *Mycobacterium tuberculosis* in synovial fluid, tissue samples, bone marrow aspirate and peripheral blood

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**Extrapulmonary tuberculosis accounts for approximately 10% of tuberculous infections ; the musculoskeletal system is involved in a small number of these (10%). Skeletal tuberculosis is an indolent disease, and diagnosis may be delayed. Conventional methods are time consuming and have a low sensitivity rate. In recent years PCR-based protocols raised hopes as a reliable and fast diagnostic tool for extrapulmonary tuberculosis. The authors report the detection of *Mycobacterium tuberculosis* complex DNA in specimens from six patients using a nested PCR protocol specific for IS6110 insertion element of *Mycobacterium tuberculosis* complex. Three men and three women are reported with ages ranging from 42 to 68 years. The sites of infection were the knee and shoulder in one case each, the hip in two cases , and the thoracic spine in two cases. Diagnosis was established within three days, and treatment was initiated promptly. PCR is a technically easy approach that can be used as a first step diagnostic tool for early recognition and treatment of bone and joint tuberculosis.**

## INTRODUCTION

Although bone and joint tuberculosis (TB) has become uncommon in the western world, it remains a serious and current problem in Asia and in developing countries (18, 19). Approximately 3.8 million new cases of both pulmonary and extrapulmonary tuberculosis, mostly emerging from developing countries (about 90%), were annually reported by the World Health Organization in the early 1990s (9). Due to delayed, inappropriate or unavailable therapy, 2 to 3 million persons are dying from tuberculosis annually (4). The recent increase in the number of tuberculous infections is directly related to the increased number of immunosuppressed patients (HIV) as well as to other factors– immigration from countries with a high prevalence of tuberculosis and social problems like poverty and drug abuse (9).

Bone and joint tuberculosis, formerly a cause of deformity and disability, must be recognised and treated early, so that destruction of joints and deformity is prevented. Approximately 10% of tuberculous infections are extrapulmonary, and 10% of these involve the musculoskeletal system. Skeletal tuberculosis is an indolent disease, and delay in diagnosis has been reported to be as long as 16 to 19 months (3). Definite diagnosis of the disease requires a positive Löwenstein culture, but these cultures are positive at a rate of 50-75% only, making bacteriologic confirmation of the disease very difficult (10). Moreover, acid-fast staining is usually negative.
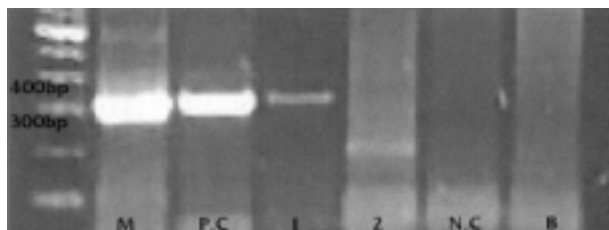
**Fig. 1.** — Electrophoresis PCR product to 1.5% Agarose Gel. **M** : marker ; **P.C** : positive control ; **1** : case No 1 ; **2** : case No 2 ; **N.C** : negative control.

In recent years, protocols based on the use of polymerase chain reaction (PCR) raised hopes for a reliable and fast diagnosis of extrapulmonary tuberculosis (8, 4). The PCR technique (fig 1) is basically a primer extension reaction for amplifying specific nucleic acids *in vitro*. The use of a thermostable polymerase allows the dissociation of newly formed complementary DNA and subsequent annealing or hybridisation of primers to the target sequence with minimal loss of enzymatic activity. PCR will allow a short stretch of DNA to be amplified to about a million fold so that one can determine its size, nucleotide sequence etc., thus providing an easier and more accurate method to detect infecting pathogens. We report the capacity for TB diagnosis by using a nested PCR protocol of specific IS6110 insertion of *M. tuberculosis* elements in specimens obtained in six patients (17).

## MATERIALS AND METHODS

Six HIV-negative patients, suffering from tuberculosis of various joints, confirmed by Löwenstein cultures, were treated in our hospital between 1996 and 2001. The knee was affected, with sinus formation, in case 1, the shoulder in case 2, the hip in cases 3 and 4, and the spine (Pott's disease) in cases 5 and 6 ; one of the spinal cases also had a sinus.

All patients had extrapulmonary TB and were meticulously examined. Tissue samples, synovial fluid, peripheral blood and bone marrow aspirate were analyed by PCR and conventional methods. There were three women and three men ; their ages ranged from 42 to 68 years (mean age : 55 years). The average duration of symptoms was 120 days (range from 30 to 395 days). Clinical signs and symptoms included swelling, pain, tenderness and limping gait. Two patients also had a dis-

charging sinus. Before attending our clinic two patients had already received inadequate, irregular and incomplete medical care, for pyogenic arthritis.

All patients were Greeks, in good condition and afebrile. None had pulmonary tuberculosis or immuno-suppression. The patients' nutritional and socio-economic status were above average. Tests such as tuberculin skin test, erythrocyte sedimentation rate, full blood count and radiographs of the chest were done routinely and were helpful but not diagnostic. The laboratory results were roughly similar in all patients. All inflammatory markers were elevated ; the Wright test was negative. No rheumatic elements were detected. The tuberculin test was negative in all cases (patients never received BCG vaccination). The sputum was negative in all patients for acid-fast bacilli and Löwenstein cultures. Malignancy screenings were unremarkable. Cultures for common bacteria were negative.

## RESULTS

Only the extrapulmonary samples are mentioned : synovial fluid, tissue specimens, bone marrow aspirate and peripheral blood (table I). Microscopic examination of smears (Ziehl-Neelsen's stain for acid-fast bacilli) was positive in cases 3 and 5. However, bacilli other than *Mycobacterium tuberculosis* might be involved. Histopathological examination showed aspecific inflammation, granulomas without central caseous necrosis, and in all cases an inner ring of epithelioid histiocytes with Langhans-type multinucleated giant cells and an outer ring of lymphocytes and fibroblasts : again, the diagnosis of tuberculosis could not be confirmed. Löwenstein cultures were positive in all six cases, which established the diagnosis of tuberculosis with certainty. Tissue samples were cultured in all six cases ; synovial fluid was cultured in cases 1 and 2. The results arrived 40 days later. Polymerase Chain Reaction was positive in all four cases (1, 2, 3 and 4) where soft tissue or synovial fluid were obtained. Bone marrow aspirate was PCR-checked in all six cases, but the test was positive in only three : cases 1, 5 and 6. Peripheral blood was PCR-checked in all cases, but the test was negative in all of them, pleading for the fact that there was no generalised tuberculosis. However, taken together all six cases were PCR

Table I. — Examination of extra-pulmonary samples

| Laboratory test | Specimen | Case<br>1 2 3 4 5 6 |
|---|---|---|
| Ziehl-Neelsen's stain | | – – + – + – |
| Histopathological examination | | ? ? ? ? ? ? |
| Löwenstein culture | Soft tissue specimen<br>Synovial fluid<br>Bone | + + + + + +<br>+ + 0 0 0 0<br>0 0 0 0 0 0 |
| Polymerase Chain Reaction (PCR) | Soft tissue and synovial fluid<br>Bone marrow aspirate<br>Peripheral blood | + + + + 0 0<br>+ – – – + +<br>– – – – – – |

+ = positive result ; – = negative result ; ? = uncertain result ; 0 = not performed.

positive : theoretically one might consider this result as a 100% sensitivity, but the number of cases was rather small. The PCR results were always reported within three days, which allowed for early treatment. Moreover, the positive PCR corroborated the clinical diagnosis in all cases.

## EVOLUTION

All patients received the triple anti-tuberculosis regime, consisting of isoniazide 300 mg/day, rifampicin 300 mg/day and ethambutol 15 mg/kg/day, with a good response (5). In the two cases with Pott's disease costotransversectomy and drainage of an abscess was performed, and in the two cases with a chronic discharging sinus surgical debridement was necessary.

PCR remained positive after therapy and turned to negative after 4 to 6 months of therapy. Clinical symptoms improved in all patients, and radiographs showed recovery.

## DISCUSSION

Tuberculosis may affect all organs. Tuberculosis of bone and joints is most often secondary to lung contamination (1, 3), which spreads via haematogenous dissemination. The spine is most often affected, followed by the hips and the knees (1, 2, 3).

Diagnosis remains difficult, partially because of the relative rarity of the bone and joint location (12, 15, 20). A quick and correct diagnosis is of great importance because of the high morbidity. Unfortunately, conventional bacteriological methods are time consuming, their sensitivity is low, and so treatment occasionally becomes empirical.

Plain radiographs are helpful, but at times it may be difficult to distinguish between tuberculous bone infection, pyogenic infection, inflammatory arthritis or bone tumor.

Biopsy has good diagnostic credit in disseminated tuberculosis, but its efficacy depends on laboratory credibility (9).

Many investigators reported that the PCR method has high specificity (92-98%) in identifying *M. tuberculosis* in various specimens including cerebrospinal fluid, pleural fluid, ascitic fluid, pericardial fluid, urine and lymph node exsudates (13, 15, 17). Although we present a small number of cases, PCR reached a 100% overall sensitivity in detecting the pathogen in extra-pulmonary specimens. This greatly exceeds the sensitivity of conventional methods of culture and of microscopy (13, 15). Furthermore, in cases where aspiration of spinal lesions is difficult, PCR analysis of bone marrow aspirate is a useful tool with higher sensitivity than that of culture, and convenient in approach (13). Although peripheral blood-based PCR assay is a good method to identify patients with active pulmonary tuberculosis, it seems that it shows low sensitivity in extra-pulmonary disease and especially in musculoskeletal tuberculosis (6). In our cases no *Mycobacterium tuberculosis* was detected in peripheral blood by PCR assay.

Molecular detection of *M. Tuberculosis* in synovial fluid and bone marrow has been reported previously as diagnostic for the disease. Only small numbers of bacilli are needed to cause tuberculosis in extra-pulmonary tissues (10) but PCR amplification of IS6110 sequence of *M. Tuberculosis* complex in synovial fluid and bone marrow renders the method capable of detecting such small numbers of bacilli in specimens in which it would otherwise be undetectable using conventional methods.

Our study confirmed that PCR facilitated the therapeutic procedure in suspected extrapulmonary

tuberculosis (11) and reduced the waiting time for diagnosis to 3 days as opposed to 40 days (7). We consider the method as a valid reliable and quick diagnostic tool, with the advantage of taking specimens from areas which are easily approachable. However, due to the small number of cases we cannot draw definite conclusions on the validity of this method. We strongly believe that prospective multicenter studies are needed in order to reach a sufficient number of patients, so that this technique can be validated for orthopaedic cases.

## CONCLUSION

*M. Tuberculosis* complex DNA was detected in all of our six cases in bone marrow aspirate or synovial fluid using PCR. It was shown that synovial fluid, tissue samples, and bone marrow aspirate are suitable sources for the diagnosis of extrapulmonary tuberculosis with PCR. The method is not only reliable but also convenient in its approach. Finally, peripheral blood-based PCR assay is a good diagnostic method for active pulmonary disease but has a poor sensitivity in extra-pulmonary disease (6). Further studies are needed in order to validate this method for orthopaedic cases.

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