Detection of *Mycobacterium tuberculosis* complex DNA in pericardial fluid, bone marrow and peripheral blood in a patient with pericardial tuberculosis

A case report

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Abstract

Definitive diagnosis of tuberculous pericarditis requires identification of bacilli in pericardial fluid or tissue. Conventional diagnostic methods are time-consuming and have a low sensitivity making bacteriological confirmation of the disease very difficult. Hereby, we report the case of molecular detection of *Mycobacterium tuberculosis* in pericardial fluid, bone marrow and peripheral blood from a 63-year-old woman with pericardial tuberculosis, using a nested PCR assay specific for IS6110 insertion element of *M. tuberculosis* complex. The patient had an excellent response to a three-drug combination anti-tuberculous regimen and 1 year later was asymptomatic, without evidence of constrictive pericarditis. © 2001 Elsevier Science B.V. All rights reserved.

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Introduction

Pericardial tuberculosis accounts for about 1–2% of all tuberculosis cases, the incidence having increased after the onset of the HIV epidemic [1]. Definitive diagnosis of the disease requires identification of bacilli in pericardial fluid or tissue [1].

Acid-fast staining is usually negative and cultures are positive in 50–75% of cases making bacteriologic confirmation of the disease very difficult [1].

In recent years, PCR-based protocols raised hopes for reliable, convenient and early diagnosis of extrapulmonary tuberculosis [2,3]. Several cases of pericardial tuberculosis have been reported in which *Mycobacterium tuberculosis* DNA was detected in pericardial effusions after pericardial puncture was performed [4,5].

Hereby, we report on molecular detection of *M. tuberculosis* in peripheral blood, bone marrow and
pericardial fluid from a patient with tuberculous pericarditis, using a nested PCR assay, specific for the IS6110 insertion element of \textit{M. tuberculosis} complex.

**Case presentation**

A 63-year-old woman was admitted to our hospital because of mild but progressive dyspnoea and low grade fever. Eight months earlier she was diagnosed as suffering from chronic idiopathic pericarditis. Methylprednisolone was prescribed and partial response was achieved.

On physical examination the patient appeared pale and dyspnoic. No rash or lymphadenopathy was found. The head and neck were normal. The lungs were clear. Cardiac sounds were diminished. No murmors or pericardial friction rub was heard. Pulsus paradoxus was absent. The liver was painful palpable 2 cm below the right costal margin. There was no peripheral edema or digital clubbing. An electrocardiogram showed a sinus rhythm with a rate of 85/min. A chest X-ray film revealed increased cardiac shadow without pleural effusion or lung infiltration. Echocardiography defined a massive pericardial effusion (Fig. 1A). A tuberculin skin test was negative and her medical history included no BCG vaccination. Anemia (Hb: 9.2 mg/dl) was found and inflammatory markers (C-reactive protein, fibrinogen, ferritin) were elevated. A test for human immunodeficiency virus (HIV) antibodies was negative. Viral serology, autoimmune and malignancy screens were unremarkable. On the fourth hospital day a dramatic increase of pericardial fluid complicated the patient illness and emergency pericardiocentesis was performed. The fluid contained an abundance of lymphocytes but microbiology and cytology were negative. At this time pericardial tuberculosis was suspected and samples from pericardial fluid were collected and analyzed by both PCR and conventional methods for the presence of bacilli, as well as peripheral blood and bone marrow aspiration smears obtained after the stabilization of patient’s illness. Concurrently, a three-drug combination anti-tuberculous regimen (isoniazide 300 mg/day, rifampicin 600 mg/day and ethambutol 25 mg/kg/day) was initiated [6]. Our PCR-based methodology detected \textit{M. tuberculosis} complex DNA in pericardial fluid, bone marrow and peripheral blood (Fig. 1C). In contrast, conventional methods failed to isolate the bacilli. She was treated for tuberculosis for 9 months (ethambutol was administered for the first 3 months) with excellent response and this confirmed our PCR results. Twelve months later she was asymptomatic without evidence of constrictive pericarditis (Fig. 1B).

**Methods**

**Acid-fast stain and bacilli culture**

All samples were investigated for the presence of acid-fast bacilli by Ziehl–Neelsen, cultured on Löwenstein–Jensen medium (bioMérieux, France) and examined histologically. Liquid culturing systems were not available in the microbiology laboratory of our hospital during that period.

**PCR protocol**

A nested PCR protocol was used in order to amplify a 316-bp fragment of IS6110 sequence of \textit{M. tuberculosis} complex, as we have previously described [7]. Briefly, after DNA isolation by lysing of mononuclear cells and phenol–chloroform extraction [7], 0.5–1 μg of extracted DNA was used in the 50-μl PCR reaction using the outer primers \textit{TJ5}, 5’-CCG CAA AGT GTG GCT AAC-3’ and \textit{TJ3}, 5’-ATC CCC TAT CCG TAT GGT G-3’ (positions 608 and 998, respectively, and amplified product 409 bp). Nested PCR was carried out in the 50-μl reaction using the inner primers \textit{OL15}, 5’-AAC GGC TGA TGA CCC AAC-3’ and \textit{STAN3}, 5’-GTC GAG TAC GCC TCC TTC TTG TT-3’ (positions 667 and 963, respectively, and amplified product 316 bp). The PCR products were analyzed in 2% agarose gel.

**Discussion**

Pericardial tuberculosis is a relatively rare disease comprising only 1–2% of all tuberculosis cases and...
may be seen in association with the pulmonary or disseminated form of the disease [1]. A rapid and correct diagnosis is of great importance because of a high incidence of constrictive pericarditis and a high mortality rate. Unfortunately, conventional bacteriological methods are time-consuming, their sensitivity is low and in most cases treatment is empirical and physicians are often uncertain whether they should continue or discontinue anti-tuberculous therapy.

Our patient was HIV seronegative, had no constitutional symptoms like malaise and night sweats and tuberculin skin test was negative. This is an unusual presentation for a patient who lives in an area with a high incidence of tuberculosis [8]. Acid-fast staining and culture of pericardial fluid failed to establish the disease but our PCR-based methodology correctly diagnosed it. Molecular detection of \textit{M. tuberculosis} in pericardial fluid has been previously reported as diagnostic of the disease [4,5]. Lower numbers of
bacilli can cause tuberculous damage in extrapulmonary disease [1] and PCR amplification of IS6110 sequence of *M. tuberculosis* complex in pericardial fluid demonstrates the validity of our method for the detection of bacilli in specimens containing numbers of bacteria undetectable by culture methods.

In our case, we detected *M. tuberculosis* complex DNA both in bone marrow and peripheral blood. Condos et al. [9] reported isolation of *M. tuberculosis* in peripheral blood from patients with pulmonary tuberculosis but this is rare in the extrapulmonary form of the disease [10]. Additionally, we have previously shown that bone marrow is a good alternative for molecular detection of *M. tuberculosis* in extrapulmonary disease [7]. The patient presented here had a long-standing disease. She was misdiagnosed as suffering from chronic idiopathic pericarditis and was under corticosteroid medication. The duration of her illness in combination with the putative immunosuppression from corticosteroids may explain the detection of *M. tuberculosis* in peripheral blood and bone marrow, as a sign of a more disseminated form of the disease. This could also explain the negative reaction of the tuberculin skin test.

In this case we demonstrated that PCR amplification of IS6110 sequence of *M. tuberculosis* complex DNA in pericardial fluid is a useful tool when pericardial tuberculosis is suspected, especially when traditional methods fail to establish the disease. Furthermore, in some cases, bone marrow and peripheral blood may constitute reliable and convenient alternative sources of specimens for such PCR analysis.

References


