

DETECTION OF MYCOBACTERIUM TUBERCULOSIS COMPLEX BY REAL-TIME PCR IN PERICARDIAL FLUID AT IBN ROCHD UNIVERSITY HOSPITAL- CASABLANCA: -A CASE REPORT-

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ABSTRACT

The diagnosis of pericardial tuberculosis may be difficult and elusive. Rapid treatment can be lifesaving but requires an accurate diagnosis. Conventional diagnostic methods are time-consuming and have low sensitivity, making bacteriological confirmation of the disease very difficult. Hereby, we report a case of a 27-year-old man who presented dyspnea with physical deterioration and anterior chest pain. The use of Real-time PCR for *M. tuberculosis* complex detection confirmed pericardial tuberculosis. The patient had an excellent response to a three-drug combination anti-tuberculosis regimen and 1 year later was asymptomatic, without evidence of constrictive pericarditis.

Keywords: Pericardial tuberculosis, MTBC, RT-PCR, IS6110

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INTRODUCTION

Tuberculosis (TB) remains a major global public health problem and one of the most frequent causes of death worldwide. The most common forms of extra-pulmonary TB (EPTB) include pleural, lymph node, osteo-articular, and abdominal TB. Other localizations such as the central nervous system, urogenital, breast, chest wall cutaneous, middle ear, and pericardial TB are rarely observed [1-3]. EPTB is often difficult to diagnose and manage. A definitive diagnosis of the disease requires the identification of bacilli in pericardial fluid or tissue [4].

Pericarditis is inflammation of the tissue that forms a sac around the heart. Many things cause heart inflammation. Common causes include viral or bacterial infections and medical conditions that damage the heart and cause inflammation.

Tuberculous pericarditis is a relatively rare disease, comprising only less than 2% of all tuberculosis cases

and may be associated with the dissemination of pulmonary form of the disease. Standard techniques are time consuming, their sensitivity is limited and the treatment is empirical, and physicians are uncertain to continue or stop the anti-tuberculosis therapy [5, 6]. Recently Molecular techniques have raised hopes for reliable convenient and early diagnosis of extra-pulmonary.

Numerous cases of tuberculous pericarditis have been reported by literature [6-8]. In this case study, we report a case of EPTB unexpectedly detected from a pericardial fluid sample using an IS6110 Real-time PCR.

PATIENT AND OBSERVATION

A 27-year-old man was admitted to Pulmonary Service at the Ibn Rochd University Hospital Center, Casablanca, after a 3-month symptomatology of aggregative dyspnea evolving with bronchial

syndrome with cough and yellow phlegm without hemoptysis in a context of physical deterioration with anterior chest pain.

On physical examination, the patient appeared pale with dyspnea. No edema of the lower limbs or clinical signs of myasthenia. The computerized tomography (CT) scan showed anterior mediastinal masses with thymic and pericardial invasion with some upper right pulmonary nodules. Flexible bronchoscopy showed the presence of white-smooth granulation on the posterior surface of the trachea, extrinsic compression of the left main bronchus with infiltration of the 2nd diffuse degrees, no sign of heart failure on cardiovascular examination. Echocardiography defined a massive pericardial effusion (**Figure 1**), measuring (RV = 42mm; Heart Apex= 36mm; LV=46mm), invagination of the wall of the right atrium (RA) and the right ventricle (RV) and significant variations in mitral and tricuspid flow with an inferior vena cava (IVC) at 25mm.

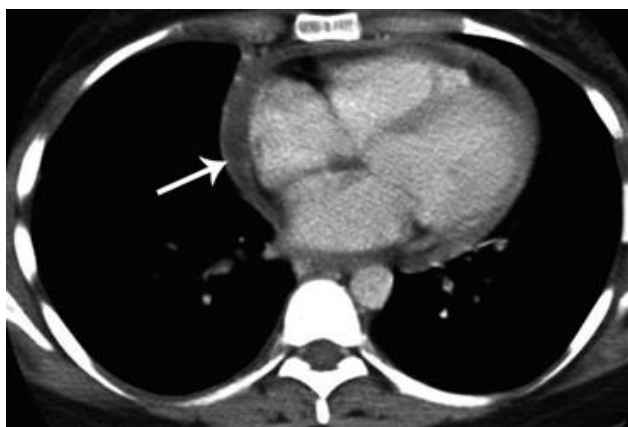


Figure 1: Axial contrast-enhanced CT scan shows pericardial effusion (white arrow)

The patient had no anemia (Hb=13.2 mg / dl), biological and inflammatory markers (CRP, ESR) were normal, medical history includes BCG vaccination at birth, Viral Hepatitis serology and human immunodeficiency virus test (HIV) were negative. The patient was under amoxicillin/clavulanic acid (1000mg/125mg) 3 times daily for 7 days without significant improvement. A dramatic increase in pericardial fluid complicated the patient's illness and emergency pericardiocentesis was performed. The pericardial fluid was sent to the laboratory for further analysis.

The pericardial fluid analysis showed the following results: proteins levels of the pericardial fluid were

47g/L, cytology showed an abundance of lymphocytes with 1200 WBC/ μ l. Sample was examined for Acid Fast Bacilli (AFB) smear by Ziehl-Neelson staining, then digested and decontaminated using conventional techniques and cultured in parallel on two Lowenstein Jensen (LJ) medium, the tubes were incubated for eight weeks at 37°C. These conventional bacteriological methods showed negative results.

The boiling method was used for sample's inactivation, extraction was carried out by Qiamp DNA Minikit (Qiagen, Hilden, Germany) according to the recommendations of the manufacturer, and 150 μ L of DNA were stored in a sterile tube at -20°C until the use by Real-time PCR. We used a duplex real-time PCR previously published [8] to detect MTBC presence. The IS6110 sequence was detected at 36 Cycle Threshold (Ct) in the pericardial fluid. The internal control was amplified and the reaction was validated.

The patient was under three-drug combination anti-tuberculous therapy (isoniazid 300 mg/day, rifampicin 600 mg/day and ethambutol 25 mg/kg/day), he was treated for tuberculosis for 9 months (ethambutol was administered for the first 3 months). Twelve months later, he was asymptomatic without evidence of constrictive pericarditis

DISCUSSION

Tuberculous pericarditis is a relatively rare disease comprising only less than 2% of all tuberculosis cases and may be associated with the dissemination of pulmonary form of the disease [6]. Standard techniques are time consuming, their sensitivity is limited and the treatment is empirical, and physicians are uncertain to continue or stop the anti-tuberculous therapy [7].

The clinical presentation of tuberculous pericarditis is variable and non-specific with symptoms including fever, night sweats, fatigue, and weight loss. The most common symptoms are cough, chest pain, and dyspnea [11, 12]. In some cases, evidence of chronic cardiac compression mimicking heart failure can be present [13]. Cardiac tamponade may present as a complication of pericardial effusion. Sometimes vague symptoms make the condition difficult to recognize. Prompt treatment of tuberculous pericarditis may be lifesaving. Effective treatment requires a rapid and accurate diagnosis, which is often difficult [14].

The patient didn't show many constitutional symptoms like night sweats or malaise which is unusual in an area where extra- pulmonary TB is

relatively high [15]. Mycobacteria can gain access to the pericardium in different ways. Most commonly this is through the mediastinal lymph nodes. Mediastinal node inflammation with subsequent central necrosis is a characteristic feature of TB. Other routes of infection include hematogenous spread, contiguous spread from the lung, the visceral pleura or a diseased rib [16].

AFB smear and culture on solid medium were not able to detect MTBC in pericardial fluid, Acid-fast staining is usually negative for pericarditis TB and cultures are positive in 50–75% of cases making bacteriologic confirmation of the disease very difficult [6] different authors approve that conventional methods are time consuming and their sensitivity is low[13,18]. Real-time PCR showed amplification of the IS6110 sequence and Ct value of 36, this low value could be explained by low numbers of bacilli in the sample and high sensitivity of this techniques compared to conventional methods. The response to therapy constituted the definitive criterion for the final diagnosis of peritoneal tuberculosis and confirmed the results of our molecular analysis. Many authors showed the effectiveness of molecular techniques in pericardial tuberculosis [16-19], but these studies need a large sample number and applicability in numerous contexts for standardization.

CONCLUSION:

This study demonstrates the first use of Real-time PCR Taqman in Morocco for pericardial TB detection. This technique is a good tool when tuberculous pericarditis is suspected after failure of conventional techniques to detect the disease.

Conflict of interest

The authors declare that there is no conflict of interest. No financial support was provided for the authors.

Abbreviations:

RT-PCR	: Real Time Polymerase chain reaction
EPTB	: Extra Pulmonary tuberculosis
MTBC	: Mycobacterium Tuberculosis
CT	: Computerized tomography
ESR	: erythrocyte sedimentation rate
WBC	: White blood cells
CRP	: C-reactive protein.
AFB	: Acid- Fast Bacilli
LJ	: Lowenstein Jensen

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