

Case Report

TB specific intracellular cytokines production in Synovial liquid for diagnosis of tuberculous arthritis

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Abstract

Introduction: Skeletal tuberculosis (TB) accounts for about 10 to 35% of extrapulmonary cases and the knee is the most frequent site after the spine and hip. The diagnosis is difficult and largely clinical.

Case presentation: This is a case of a young Pakistani man with a history of joint pain for about 4 years, who was diagnosed with chronic arthritis of the right knee. Microscopy of synovial fluid and conventional diagnostic tests to identify *Mycobacterium tuberculosis* were negative, while a non-classical method based on intracellular cytokine flow cytometry response of CD4 T-cells in synovial fluid helped us to address the diagnosis, which was subsequently confirmed by Polymerase Chain Reaction (PCR).

Conclusions: Thanks to an innovative immunological approach, supported by PCR for detection of *M. tuberculosis* DNA, we were able to diagnose tuberculous arthritis of the knee, which allowed prompt initiation of treatment to reduce morbidity and mortality.

Key words: Tuberculous arthritis; TB diagnosis; intracellular cytokines.

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Introduction

Tuberculosis (TB) is still a major public health problem in both developing and industrialized countries. Although pulmonary TB plays the most important role due to its relevance to public health, extrapulmonary tuberculosis (EPTB) such as osteoarticular TB is also relevant. TB arthritis accounts for approximately 10-11% of extrapulmonary cases and approximately 1-3% of all cases of tuberculosis [1].

TB arthritis is usually monoarticular, primarily involving large weight-bearing joints, with the knee and hip being the most commonly affected areas. It is typically the result of a direct hematogenous spread of mycobacteria from visceral foci such as the lungs, kidneys, and lymph nodes [2].

Osteoarticular TB often starts as synovitis progressing to periarticular demineralization, marginal erosions, and finally joint destruction. An early diagnosis is very important to ensure full recovery with near-normal function [3]. The delay in diagnosis is mostly due to under-recognition of disease and

diagnostic difficulties, because of the low sensitivity of traditional diagnostic methods, resulting in higher morbidity and mortality.

We present a case study of a person with TB osteoarthritis who experienced a delay in diagnosis of about 4 years and in whom an innovative immunological flow cytometry test confirmed the clinical diagnosis.

Case Report

A 36-year-old Pakistani man with normal weight, living in Italy for 9 years, was referred to our clinic for chronic right knee pain starting 4 years before presentation. The patient had no significant past medical history. He worked as a gardener, and due to chronic pain, he frequently self-administered non-steroidal anti-inflammatory drugs. The family doctor suspected inflammatory arthritis. There was no specific history of trauma. Four months prior to his clinic appointment he underwent arthrocentesis with synovial fluid (SF) culture, showing growth of oxacillin-resistant

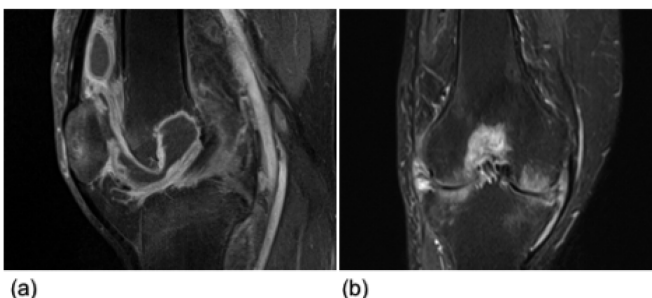
Staphylococcus haemolyticus. No further investigations were performed on SF. Based on the antimicrobial susceptibility testing, he was started on levofloxacin 750 mg/day and rifampicin 600 mg/day, with no major improvement. He underwent Magnetic Resonance Imaging (MRI) of the knee, which showed extensive destructive intra-articular changes (Figure 1 A, B). When the patient was assessed in our clinic, a physical examination revealed a hot, swollen right knee, with a reduced range of motion. The patient could not recall whether he had previously been vaccinated with BCG. Blood tests showed normal white blood cells count, slightly decreased haemoglobin (11.9 mg/dL), erythrocyte sedimentation rate of 80 mm/h (cut off < 20 mm/hour), and C-reactive protein (CRP) of 30.5 mg/L (cut off 10 mg/L). He underwent a Tuberculosis Skin Test (TST) which resulted positive, while a chest X-ray did not show any abnormalities. Arthrocentesis was performed again. SF had a yellowish, turbid appearance, with increased protein level at 5 g/dL (range 1-3), $7.120 \times 10^3/\mu\text{L}$ white cells (87% neutrophils). Gram and Ziehl-Neelsen stains, as well as culture for common pathogens, were negative. Qualitative Polymerase Chain Reaction (PCR) for *M. tuberculosis* was negative, and culture for TB and biopsy for histopathological examination were sent to the laboratory. In order to help with the diagnosis of TB, we performed an alternative immunological method based on multifunctional T-cells, which has been suggested in recent years as a new tool for the differential diagnosis between active and latent TB infection (LTBI). In a previous study [4] we used an intracellular cytokine flow cytometry (ICCFC) protocol to assess mono-functional and multi-functional MTB-

specific CD4+ T-cells in peripheral blood, and we proposed an immune-based approach, which could improve rapid differentiation between patients with no TB infection or active/latent TB. Although the original study involved pulmonary TB, we previously applied the same method to the diagnosis of TB meningitis [5] and explored its feasibility in TB arthritis in the case presented here. The same method of staining of peripheral whole blood (WB) was applied to synovial fluid. Briefly, SF (0.5 mL) was added to the 4 tubes of QFT Gold Plus (QFT-Plus) containing saline solution (negative control), phytohemagglutinin (positive control), TB1 and TB2 TB-specific antigen tubes. The TB1 tube detects a specific CD4+ T-cell response, while TB2 detects CD4+ and CD8+ T-cell responses [6]. A co-stimulation with $5\mu\text{L}/\text{mL}$ anti-CD28 plus anti-CD49d (BD Bioscience, Pharmingen, Italy) and $10\mu\text{g}/\text{mL}$ BrefeldinA (Sigma-Aldrich) was added to all tubes. After 18 hours of incubation, the cell surface staining was performed with the markers anti-CD45-VioBlue and anti-CD4 PE-Vio770 (Miltenyi Biotec, Germany), then the cells were lysed, permeabilized, and intracellularly stained with anti-IFN- γ FITC, anti-TNF- α APC, and anti-IL-2 PE (Miltenyi Biotec, Germany). Finally, samples were processed using a MACSQuant Analyzer flow cytometer (Miltenyi Biotec, Germany) and analyzed with FlowJo Software version 10, as previously described [4].

Figure 2 shows the levels of IFN- γ , IL-2 and TNF- α produced by CD4+ T-cells in WB (a) and SF (b). We classified T-cells producing any of the 3 cytokines (IFN- γ or IL-2 or TNF- α) as “activated T-cells,” while “polyfunctional T-cells” were those expressing all 3 cytokines simultaneously. The percentage of activated CD4+ T-cells was elevated in both WB (0.56%) and SF (20.27%). Using a cut-off of > 0.45%, the test was considered to be consistent with TB infection [4]. The percentage of polyfunctional CD4+ T-cells was 0.118% in WB and 5.42% in SF. Based on a cut-off of < 0.182%, the assay was considered to be positive for active TB in WB. A higher proportion of polyfunctional CD4+ T-cells was detected in SF, indicating a strong response to TB antigens locally. So far, little data is available on how to interpret ICCFC results on biological samples other than blood, but a higher percentage of polyfunctional CD4+ T-cells could be a consequence of a higher immunological response to TB antigens in the SF, which would point towards a diagnosis of tuberculous arthritis.

Interestingly, PCR for *M. tuberculosis* resulted negative on the same SF sample. The biopsy showed chronic inflammation, with non-necrotizing epithelioid

Figure 1. (a) Knee MRI with extensive articular morphostructural alteration characterized by marked and severe hyperplasia of the synovial cloth that extends to the level of the quadricipital recess, associated with a minimum portion of joint effusion largely organized; presence of extensive erosive changes with loss of bone substance at the level of the tibial plateau of both femoral condyles; (b) After administration of the contrast agent, synovial strengthening was observed that involved adjacent tissues.



granulomas. The patient underwent a repeat arthrocentesis one week after the previous one, and PCR for *M. tuberculosis* resulted positive on the SF sample.

The patient was started on an anti-TB regimen including isoniazid 300 mg QD PO, ethambutol 400 mg PO TID, rifampicin 600 mg intravenous (IV) QD, and pyrazinamide 500 mg PO TID, as well as intravenous steroids (iv methylprednisolone 40 mg/die), which were gradually tapered (over 6 weeks).

After 18 months of anti-TB therapy, progressive functional improvement was observed locally, with an increased range of motion.

Discussion

Skeletal TB is a relatively rare extrapulmonary complication of *Mycobacterium tuberculosis* resulting from hematogenous spread of TB bacilli. Most of the time, the primary focus of infection remains unidentified (50%), while spread from a pulmonary or genitourinary focus is observed in 30% and 20% of cases, respectively [3].

Osteoarticular TB has been most commonly reported to affect large, weight-bearing joints, like hip and sacroiliac joints, followed by knees, and it is usually monoarticular [7].

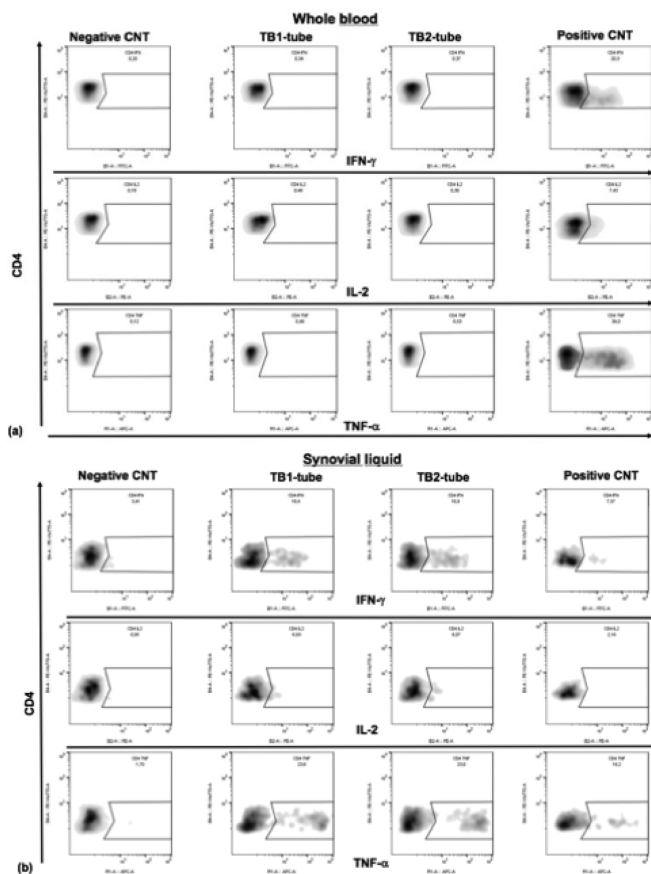
We report a case of TB arthritis in a young Pakistani male without comorbidities, who attended our clinic with chronic pain and swelling of his right knee for the previous 4 years. Initial investigations were inconclusive and did not support a diagnosis of TB osteoarthritis. In particular, both the Ziehl-Neelsen stain and TB culture were negative. In addition, molecular tests using PCR to detect *Mycobacterium tuberculosis* were also negative on the initial SF sample.

Given the high clinical suspicion for TB osteoarthritis, we used an innovative assay to detect CD4+ T-cells intracellular response to TB antigens in SF, as previously applied to whole blood [4]. In this case study, the results of this assay assisted us in the diagnosis of osteoarticular TB, which was confirmed by positive PCR for *M. tuberculosis* on repeat SF sample. This was based on the hypothesis that mononuclear cells localized to infected sites produce more cytokines in response to TB antigens than peripheral blood mononuclear cells, as previously demonstrated in other studies [17]. Antigen-specific effector memory T-lymphocytes migrate to the site of inflammation in active TB and rapidly release T-helper cell type-1 cytokines following antigen stimulation [18].

In the assay used in this case, intracellular response in WB suggested active TB infection, as shown by the polyfunctional CD4+ T cell activation, based on a previously proposed algorithm [4]. The same test performed in SF confirmed active *M. tuberculosis* infection with a high local immune response. In SF the increased proportion of CD4+ T-cells producing cytokines could reflect the high number of mononuclear cells localized to infected sites. A similar phenomenon was described for other forms of EPTB such as in pleural fluid, alveolar fluid, and in cerebrospinal fluid in TB meningitis [5,16,19,20]. Early diagnosis and treatment of TB arthritis are essential to preserve the articular cartilage, joint space, and joint function [8].

Although prompt and rapid identification of TB arthritis is crucial for successful disease management,

Figure 2. Flow cytometry analysis of CD4+ T-cells of whole blood (a) and synovial fluid (b) after stimulation with TB antigens. The gating strategy excluded debris and identified CD4+ on CD45+ lymphocytes. The subsequent analysis was on CD4+ gate to describe IFN- γ , IL-2, and TNF- α total producing T-cells on WB (a) and on SF (b) produced in the negative control (from left to right), TB1, TB2, and mitogen (positive control). On the plot, the percentages of the different population cells, calculated by FlowJo Software version 10 were shown and were defined in CD4+ cell gate based on total IFN- γ , IL-2, and TNF- α production.



in most cases, diagnosis is significantly delayed, which is often attributable to lack of awareness, insidious onset, absence of characteristic early radiographic findings, and often absence of constitutional symptoms or concomitant pulmonary involvement. Up to 80% of patients with tuberculous knee arthritis are misdiagnosed before surgery with rheumatic arthritis, gout, or osteoarthritis [9].

In our case report, initial investigations on SF, including culture, were negative. It is important to note that TB arthritis is a paucibacillary form of tuberculosis, which makes the isolation of *M. tuberculosis* on SF samples quite challenging [10].

In the last decades, nucleic acid-based amplification (NAA) tests have emerged as important tools for diagnosing TB. Unfortunately, these tests have a high specificity (100%) but low sensitivity (32%-78%) [11-13]. In our patient, the first PCR test was negative, perhaps due to the small volume extracted (3 mL). The results of the assay described earlier prompted us to perform a repeat arthrocentesis, which yielded an adequate volume of SF (10 mL) on which the diagnosis of TB osteoarthritis was confirmed with molecular testing.

The use of interferon-gamma release assay (IGRA) has recently shown promising results in diagnosing active extrapulmonary TB [10-14], with a sensitivity of 67%-93% and a specificity of 69%-78% on peripheral blood samples [15,16].

Conclusions

We present a case of osteoarticular TB where the use of an innovative immunological approach, based on the detection of local response to TB antigens in SF, allowed us to promptly diagnose osteoarticular TB of the knee. Large prospective studies are warranted to confirm the role of IGRA in the diagnosis of osteoarticular TB.

Ethics approval and consent to participate

Written informed consent for publishing clinical details and images was obtained from the patient. Ethical approval to report this case was not required.

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