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CASE STUDY

Re-activation of bovine tuberculosis in a patient treated with infliximab

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ABSTRACT: Treatment with tumour necrosis factor-α inhibitors increases the risk of tuberculosis (TB). Screening for latent TB infection (LTBI) and prophylactic treatment has become mandatory.

A 79-yr-old female with a history of severe erosive sero-positive rheumatoid arthritis was screened for LTBI before initiation of treatment with infliximab. The tuberculin skin test (TST) was negative, chest radiography was normal and she had no known risk factors for TB. After 4 months of treatment with infliximab, the patient developed ascites caused by *Mycobacterium bovis*. The TST was repeatedly negative. QuantiFERON®-TB (QFT) testing performed during screening and immunosuppressive treatment was indeterminate, whereas the QFT test performed at the time of ascites puncture was positive.

The patient history revealed previous work at a dairy, with probable exposure to unpasteurised milk from *M. bovis*-infected cattle.

Re-activation of bovine tuberculosis is a risk in people with recent or previous exposure to unpasteurised dairy products. The QuantiFERON®-TB test has the potential to detect *Mycobacterium bovis* infection. Indeterminate test results reflect either anergy, due to poor immunity, or technical problems and should be cautiously interpreted and as a minimum be repeated. Studies are ongoing to determine the role of QuantiFERON®-TB testing in the screening for latent tuberculosis infection.

KEYWORDS: Diagnosis, immunosuppression, interferon-γ release assays, *Mycobacterium bovis*, tuberculosis, tumour necrosis factor-α inhibitor

In the field of rheumatology, biological drugs including tumour necrosis factor (TNF)-α inhibitors have had a great impact on the treatment of resistant rheumatoid arthritis (RA). It has been shown that treatment with these biological drugs increase the risk of re-activation of latent tuberculosis (TB) infection (LTBI) [1]. Therefore, screening for LTBI and prophylactic treatment has become mandatory [2]. Screening for LTBI is compromised by low specificity of the tuberculin skin test (TST) in bacille Calmette–Guerin-vaccinated patients and low sensitivity of this test in patients with downregulated cell-mediated immunity [3, 4]. During recent years, *Mycobacterium tuberculosis*-specific interferon-γ release assays (IGRA) have been shown to be an asset in screening for LTBI and to be more sensitive in immunocompromised individuals [3, 5–8]. The evaluation of the use of IGRA as a screening tool before initiating TNF-α inhibitors is ongoing, but few data have been published [5–9]. The authors have previously reported active TB in a patient who was TST negative but IGRA positive before intensive medical immunosuppression [10], whereas another report described a patient, negative by both TST and IGRA, who developed active TB after immunosuppression [11]. To the current authors' knowledge, no reports on re-activation of *M. bovis* after TNF-α inhibition and the potential role of IGRA testing have been published.

CASE REPORT

In September 2006, a 79-yr-old female was admitted to the Dept of Infectious Diseases, Hvidovre, Denmark. She had a history of severe erosive sero-positive RA for ~40 yrs. She had been treated with gold, penicillamin and sulfasalazin, and for the past 10 yrs with methotrexate and prednisolone.

In May 2005, she was screened for LTBI before initiation of treatment with infliximab. The TST was negative, chest radiography was normal and she had no known risk factors for TB. In October

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2005, the patient began treatment with infliximab. There was clinical improvement and four series of treatment were given. In March 2006, she complained of a dry cough for 2 weeks, night perspiration and a weight loss of 9 kg, but no fever; infliximab treatment was interrupted. Chest radiography showed pleural effusion on the right side and 1.5 L clear fluid was evacuated. A computed tomography (CT) scan of the thorax showed multiple small nodules. The pleural fluid was negative by microscopy, culture and PCR for *M. tuberculosis* complex and no explanation was found for this effusion. Due to spontaneous recovery, infliximab treatment was resumed in June 2006 and two series of treatment were administered. By August 2006, the patient complained of fever, loss of appetite and nausea. A CT scan showed ascites and unaltered chest nodules. The TST was repeatedly negative. The ascitic fluid was positive by PCR for *M. tuberculosis* complex and when cultured grew *M. bovis*. The patient history additionally revealed that she had worked at a dairy during her childhood and had probably been exposed to unpasteurised milk from *M. bovis*-infected cattle. The patient was treated with isoniazid, rifampicin, ethambutol and pyridoxin for 9 months as *M. bovis* is naturally resistant to pyrazinamide. At present the patient is well and infliximab treatment is scheduled to be restarted.

Before initiation of infliximab treatment, during treatment with methotrexate and prednisolone, a second generation QuantiFERON®-TB Gold (QFT-G; Cellestis, Carnegie, Australia) test was performed and results were indeterminate (table 1). Given the negative TST, normal chest radiography and no TB exposure, no further action was taken. During the investigation for peritoneal *M. bovis*, the whole-blood test was repeated using the third generation QFT-G in-tube (QFT-GIT) test and was found to be positive (table 1).

TABLE 1 Interferon-γ levels found by QuantiFERON® testing

	QFT-G	QFT-GIT
PHA	0.27	0.41
ESAT-6	0.03	
CFP-10	0.08	
ESAT-6/CFP-10/TB7.7		1.21
Result	Indeterminate	Positive

Data are presented in $IU \cdot mL^{-1}$. For the QuantiFERON®-TB Gold (QFT-G) test (Cellestis, Carnegie, Australia) performed in May 2005, 6 mL whole blood was drawn into a heparinised blood collection tube and transported to the National Reference Laboratory for Mycobacteriology at the Statens Serum Institute (Copenhagen, Denmark). The blood samples were divided into four aliquots and stimulated in a 24-well culture plate (Nunc, Roskilde, Denmark) with saline, phytohaemaglutinin (PHA) or *Mycobacterium tuberculosis*-specific peptides (early secreted antigenic target (ESAT)-6 or culture filtrate protein (CFP)-10). For the QFT-G in-tube (QFT-GIT) test performed in September 2006, three whole-blood samples, each 1 mL, were drawn directly into vacutainer tubes coated with saline, PHA or *M. tuberculosis*-specific peptides (ESAT-6, CFP-10 and TB7.7). The tubes were incubated at the hospital for 18 h at 37°C and the plasma was harvested. ELISA was performed according to the manufacturers' instructions and software.

DISCUSSION

Re-activation of *M. tuberculosis* is well known [1, 2] and rare cases of mycobacteria other than tuberculosis (MOTT) have been reported in patients treated with TNF-α inhibitors [12]. The first case of re-activation of *M. bovis* infection during TNF-α inhibition is reported here. Bovine TB can be transmitted either by drinking raw milk from infected animals or in some cases by inhalation of infected aerosols.

The patient had no known risk of infection with *M. tuberculosis*, whereas the history revealed that she could have been infected with *M. bovis* during childhood. Normal screening procedures using chest radiography, TST and case history failed to identify her as a high-risk patient. The TST should have been positive in a patient infected with *M. bovis*, but it is well known that the TST can be falsely negative in immunocompromised patients, as in this case.

IGRA can potentially detect latent bovine TB since the antigens used are also present in *M. bovis*, and IGRA are actually used for the diagnosis of *M. bovis* infection in cattle [13]. In contrast, the antigens used are not found in most MOTT, so IGRA will not be useful for the diagnosis of MOTT infections. In the present case, the first QFT-G test was indeterminate whereas the QFT-GIT test was positive when the patient was later diagnosed with disease due to *M. bovis*. The initial indeterminate QFT-G test was not repeated and it was not possible to determine whether the indeterminate test was due to T-cell anergy, technical problems or batch variation. The QFT-GIT test is potentially more sensitive than the heparin version due to an additional antigen (TB7.7) and possibly due to immediate antigen and mitogen contact in the tube.

It is well documented that the number of indeterminate QFT test results is increased in severely immunocompromised patients [3, 9]. Enzyme-linked immunosorbent spot assays may be more sensitive than the whole-blood assay due to standardisation of lymphocyte number during incubation. Comparative studies are ongoing. The IGRA tests are reported to be more sensitive than the TST [3, 6, 7, 9, 10] in immunocompromised patients, but there is still a concern that sensitivity is suboptimal. Ways to increase sensitivity of the IGRA are currently being explored and sensitivity could be enhanced by, for example, lowering the cut-off point for a positive IGRA result in immunocompromised individuals in line with the TST guidelines [2] or by including alternative or additional biomarkers, such as interferon-γ inducible protein 10 [14].

In conclusion, re-activation of bovine tuberculosis is a risk in people with recent or previous exposure to unpasteurised dairy products or animals with bovine tuberculosis. The interferon-γ release assays are highly specific for mycobacteria belonging to the *Mycobacterium tuberculosis* complex, which includes not only *Mycobacterium tuberculosis* and *Mycobacterium Africanum* but also *Mycobacterium bovis*. Thus, these tests have the potential to detect active and latent *Mycobacterium bovis* infection. Indeterminate QuantiFERON®-TB test results should be interpreted cautiously in immunocompromised individuals and the test should, as a minimum, be repeated to rule out technical errors and negative results should be interpreted with caution. Screening with both the tuberculin skin test and interferon-γ release assays may increase sensitivity. Further

studies on the use of interferon-γ release assays as a screening tool for tuberculosis before tumour necrosis factor-α inhibition are ongoing.

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