Case Report

Temporal interferon-gamma release response to *Mycobacterium kansasii* infection in an anorexia nervosa patient

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Due to the differences in the management of *Mycobacterium kansasii* disease and tuberculosis, an accurate diagnosis is required. This report, which describes what we believe to be the first documented case of *M. kansasii* infection in a patient suffering from anorexia nervosa, sheds light on the possible occurrence of a non-tuberculous mycobacterial infection that can mimic tuberculosis, on the risk of a misleading interpretation of interferon-gamma release assays, and on the temporal response to these tests.

Introduction

Specificities in the case management of *Mycobacterium kansasii* disease and tuberculosis (TB) require accurate differential diagnoses. This report, which describes what we believe to be the first documented case of *M. kansasii* infection in a patient suffering from anorexia nervosa (AN), sheds light on the occurrence of an infection caused by non-tuberculous mycobacteria (NTM) that can mimic TB and on the usefulness of methods that allow accurate diagnostics.

Case report

A 20-year-old female patient was admitted in April 2009 for a cough lasting for more than 2 weeks in the absence of fever, night sweats or haemoptysis, but with the presence of a 25-mm-diameter cavity in the superior lobe of the right lung on the chest radiography and computerized tomography (CT) scans. The patient was born in France and had had no serious illness before the diagnosis of AN at the age of 18 (American Psychiatric Association, 2000). The patient had progressively regained weight in the following year, with an increase in her body mass index (BMI) from 14.5 to 18 kg m\(^{-2}\) at the time of admission. She had been routinely vaccinated with BCG at the age of 1 month, and systematic school monitoring showed iterative negative tuberculin skin tests (TSTs) until the age of 11. Upon admission, a positive TST (Tubertest; Sanofi Pasteur MSD) was immediately confirmed by a positive interferon (IFN)-gamma release assay (IGRA) response (QuantiFERON-TB Gold In-Tube; Cellestis) (Fig. 1a). Laboratory tests revealed a white blood cell count of 3800 m\(^{-3}\), haemoglobin concentration of 120 g l\(^{-1}\) and a platelet count of 173 \(\times 10^3\) m\(^{-3}\). Serum levels of sodium (142 mmol l\(^{-1}\)), potassium (3.9 mmol

Abbreviations: AN, anorexia nervosa; BMI, body mass index; CT, computerized tomography; IFN, interferon; IGRA, interferon-gamma release assay; NTM, non-tuberculous mycobacteria; TB, tuberculosis; TST, tuberculin skin test.
that time showed normal serum levels of immunoglobulins was discontinued. Immunological investigation performed at 2007 American Thoracic Society/Infectious Diseases Society of America criteria (Griffith et al., 2007) and pyrazinamide reassessed as 

displayed low-level resistance to isoniazid. Diagnosis was 
strain was susceptible to rifampicin and ethambutol, and 
deprecated, although according to local policy, were not submitted to genomic amplification-based assays for TB. Although the patient did not present with increased risk factors for TB such as spatial proximity to a patient with infectious TB or facilitating co-morbidities, the conjunction of clinical, radiological and biological findings led to the diagnosis of pulmonary TB and to the onset of anti-TB four-drug therapy associating daily isoniazid, rifampicin, ethambutol and pyrazinamide. Within 8 to 15 days, four of the nine smear-negative clinical samples (three gastric washings and one bronchial aspirate) yielded growth of nontuberculous mycobacteria in liquid (MGIT; BD) and on solid (Coletos; Bio-Rad) culture medium incubated at 37 °C, which were identified as M. kansasii with the Genotype-CM PCR-hybridization assay (BioCentric). The strain was susceptible to rifampicin and ethambutol, and displayed low-level resistance to isoniazid. Diagnosis was reassessed as M. kansasii pulmonary infection following the 2007 American Thoracic Society/Infectious Diseases Society of America criteria (Griffith et al., 2007) and pyrazinamide was discontinued. Immunological investigation performed at that time showed normal serum levels of immunoglobulins with IgG at 9.9 g l⁻¹ (normal range 6.8–15), IgA at 0.83 g l⁻¹ (0.75–3.75) and IgM at 1.81 g l⁻¹ (0.4–2.2). Immunophenotyping of lymphocytes (1700 μl⁻¹) showed no abnormalities, with 78.9 % of CD3⁺, 46.6 % of CD3⁺/CD4⁺, 28.3 % of CD3⁺/CD8⁺, 11.4 % of CD19⁺ and 6.5 % of CD3⁻/CD56⁺. Exploration of the interleukin-12/IFN-γ axis on whole blood cells was normal (Feinberg et al., 2004). Although not recommended, since this compound in combination with rifampicin is reported to be efficient against M. kansasii strains that show a low level of resistance (Griffith et al., 2007), isoniazid was stopped after 5 months. The non-standard rifampicin–ethambutol regimen was continued for a total of 18 months (i.e. the recommended 12-month period of negative sputum samples). The patient progressively regained body weight and the favourable outcome was confirmed with a CT scan 1 year later (Fig. 1c) and a normalized BMI (20.5 kg m⁻²) after 18 months. Monitoring of the IGRA response showed decreasing values, from 1.09 IU ml⁻¹ at the onset of treatment to levels below the positivity threshold after 5 months (Fig. 1a). The search for the infecting strain in the direct environment of the patient was unsuccessful, although investigation of water taps at the patient’s place of residence yielded the growth of environmental mycobacteria that were not identified as the M. kansasii species.

Discussion

We believe that this is the first report of M. kansasii infection in a patient suffering from AN, although some cases of TB or other NTM disease have been sporadically reported. M. kansasii disease, the second most common cause of NTM disease in the USA, sometimes mimics TB, but no specific clinical symptom, radiological sign or biological test for either disease has been identified so far (Shitrit et al., 2007). Specificities in case management, involving contact-tracing measures in the case of TB, strengthen the need for accurate differential diagnosis of both diseases.

In addition to direct diagnostic techniques using culture or genomic amplification, T-cell-based IGRA have been developed as alternative tools to the TST for the indirect diagnosis of TB (Mori, 2009). The improved specificity of IGRA for TB, compared with the TST, and previous reports of either TB or NTM infections in AN patients, led to the carrying out of an IGRA immediately upon the positive TST result. The enhanced specificity of these assays relies on the use of a combination of recombinant early secretory antigen target (ESAT)-6, culture filtrate protein

Fig. 1. Time-course of IGRA and CT scans during treatment of an M. kansasii pulmonary infection in a patient presenting with AN. (a) IGRA (QuantiFERON-TB Gold In-Tube) was monitored for 17 months (filled line), starting at the time of diagnosis (0 months) and onset of anti-mycobacterial treatment. Although of moderate intensity (maximum concentration of 1.09 IU IFN-gamma ml⁻¹), the IGRA response had decreased to levels below the negative threshold (dotted line) within 5 months. (b, c) Thin-section CT scan of the right lung at the time of admission (b) and 1 year later (c). Within the first year of treatment, the thin-walled cavity in the upper lobe of the right lung (b, white arrow) evolved to a fibrotic cicatricial pattern (c, black arrow).
(CFP)-10 and TB7.7 M. tuberculosis antigens for the QuantiFERON-TB Gold In-Tube assay. The esat-6 and cfp-10 genes are located on the genomic region of differentiation 1 (RD1) of M. tuberculosis. However, NTM phylogenetically related to M. tuberculosis, such as M. kansasii, Mycobacterium marinum, Mycobacterium szulgai, Mycobacterium riyadhense and Mycobacterium gastri, also carry RD1 regions with variants of esat-6 and cfp-10 genes (Devulder et al., 2005; Gey van Pittius et al., 2006; van Ingen et al., 2009). For M. kansasii, ESAT-6 and CFP-10 antigens were observed in both clinical and environmental strains and they were responsible for T-cell cross-reactivity and positive IGRA responses (Arend et al., 2005; Kobashi et al., 2009a, 2009b). This case thus illustrates a limit in the specificity of IGRA due to the presence of shared antigens in several mycobacterial species, and strengthens the essential role for direct diagnostic methods, such as culture, for accurate differential diagnosis of TB and M. kansasii infection.

Cross-reactivity of IGRA for TB and M. kansasii infection raises the point whether the observed IGRA response was due to a hypothetical coincidental latent TB rather than to M. kansasii infection. Concomitant infection by M. tuberculosis and M. kansasii is rare. This patient was considered to be at low risk of acquiring TB, since she was living in a region of low TB incidence (8.1/100 000 in France in 2010) (Antoine & Che, 2012), did not have a low socioeconomic background and had no known history of close contacts with TB. In the context of AN, low body weight and malnutrition are predisposing factors for TB in the presence, unlike this patient, of long-standing, severe emaciation (Pertschuk et al., 1982). More generally, unlike other forms of starvation and despite the paucity of clinical symptoms that renders diagnosis more difficult, the incidence of bacterial infection does not appear to be increased in AN patients (Bowers & Eckert, 1978; Brown et al., 2005; Hütter et al., 2009). Taken together, the likelihood that the positive IGRA response in this patient is due to latent TB rather than to M. kansasii infection is low.

The decreasing kinetics observed for the IGRA response upon treatment of M. kansasii disease were similar to those reported for TB or latent TB, although this pattern was not observed in all studies (Bocchino et al., 2010; Carrara et al., 2004; Chiappini et al., 2012; Katiyar et al., 2008; Kobashi et al., 2009a; Mori, 2009; Pai et al., 2006). Several hypotheses were raised for these discrepancies, such as reactivation in high burden settings, persistence of effector T-cells and within-subject variability (Chiappini et al., 2012; Ewer et al., 2006; Pai et al., 2006; van Zyl-Smit et al., 2009). IGRA's, such as QuantiFERON-TB Gold In-Tube, are not recommended for the routine monitoring of TB medical treatment but this similarity suggests that the evolution of immunity during anti-mycobacterial treatment is similar for both types of infection. Further studies are needed to investigate whether, for M. kansasii disease, the decrease in the QuantiFERON-TB Gold In-Tube assay response should be interpreted as a sign of treatment efficacy.

In conclusion, this case of M. kansasii pulmonary infection in an AN patient highlights the possible occurrence of an NTM infection that can mimic TB and strengthens the need for clinicians and microbiologists to acknowledge the limitations of IGRA's in regard to specificity among mycobacterial infections and, therefore, reinforce the central role of direct diagnostic methods for the accurate diagnosis of mycobacterial diseases.

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References

association with the duplication of the ESAT-6 (esx) gene cluster regions. *BMC Evol Biol* 6, 95.


