

Case Report

Immune reconstitution inflammatory syndrome due to *Mycobacterium genavense* in an HIV-infected patient: Impact of rapid species identification by *rpoB* sequencing on clinical management

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1

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Introduction: Diagnosis of infections due to *Mycobacterium tuberculosis* has been recently facilitated with the worldwide implementation of rapid molecular tests. However, the diagnosis of non-TB mycobacteria (NTM) remains a major challenge for laboratories, as this group of bacteria contains over 150 different species with highly variable bacteriological properties. The development and implementation in clinical practice of techniques allowing rapid identification of NTM at the species level is, therefore, important for clinical laboratories. We report the case of an HIV-1 positive patient presenting with severe NTM infection who benefited from a novel rapid diagnostic technique, based on the direct sequencing of the *rpoB* gene from smear-positive clinical samples.

Case Presentation: A 43-year-old HIV-1 positive woman was hospitalized for bicytopenia in the presence of an inflamed supraclavicular lymph node. The diagnosis of disseminated *Mycobacterium genavense* infection, which involved the bone marrow and several lymph nodes, was missed by conventional mycobacteriological techniques. Identification was obtained by performing direct sequencing of the *rpoB* gene.

Conclusion: We describe a novel technique that allows the rapid diagnosis of NTM infections directly from biological samples. This technique is now implemented in our routine workflow for smear and culture-positive samples, after the exclusion of TB, by rapid molecular assays.

Keywords: ART; HIV; IRIS; *Mycobacterium genavense*; NTM; rapid diagnosis.

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Introduction

The diagnosis of infections due to *Mycobacterium tuberculosis* (Mtb) has made an unprecedented step forward recently with

the worldwide implementation of rapid molecular tests. However, the diagnosis of non-TB mycobacteria (NTM) remains a major challenge for laboratories, as this group of bacteria contains over 150 different species presenting with variable bacteriological properties and clinical significance. Current molecular techniques lack sensitivity and are unable to distinguish mycobacterial species or subspecies to a level that would guide therapeutic management. Development of techniques allowing rapid identification of NTM at the species level is, therefore, important for clinical laboratories.

†These authors contributed equally to this paper.

Abbreviations: ART, antiretroviral therapy; FDG PET CT, fluorodeoxy-glucose positron emission tomography computed tomography; HIV, human immunodeficiency virus; IRIS, immune reconstitution inflammatory syndrome; Mtb, *Mycobacterium tuberculosis*; NTM, non-TB mycobacteria; TB, tuberculosis; VL, plasma viral load.

We report the case of a patient presenting with disseminated infection due to *Mycobacterium genavense*, a rare and hardly cultivable mycobacterial species. The patient benefited from direct sequencing of the bacterial *rpoB* gene from smear-positive clinical samples.

Case Report

Here, we describe the case of a 43-year-old woman, hospitalized for bicytopenia, in the presence of an inflamed supraclavicular lymph node.

The patient was diagnosed with human immunodeficiency virus-1 (HIV-1) infection nine years previously and had been treated with antiretroviral therapy (ART) containing abacavir/lamivudine and nevirapine for the past six years. She was then lost to follow up for three years and ART was interrupted.

The patient was admitted for *Pneumocystis jiroveci* pneumonia. Her CD4 cell count was $4 \mu\text{l}^{-1}$ with a plasma viral load (VL) of $273.423 \text{ copies ml}^{-1}$. Antiretroviral therapy containing tenofovir/emtricitabine and dolutegravir was begun.

Two months later, she was readmitted for fever, chills and night sweats. Clinical examination revealed an inflamed supraclavicular lymphadenopathy. The CD4 cell count was $66 \text{ cells } \mu\text{l}^{-1}$, and VL was $144 \text{ copies ml}^{-1}$. Further blood tests revealed anaemia (Hb: 10.8 g l^{-1}) a low white blood cell count ($3030 \text{ leukocytes } \mu\text{l}^{-1}$) and C-reactive Protein at 56 mg l^{-1} (NI value: $<5 \text{ mg l}^{-1}$).

A bone marrow aspirate was performed to exclude a haematological neoplasm. Cytological examination showed more than 60 % reactive plasmocytes locally, rare

epithelioid granulomas and a single Langhans giant cell with no clear cell border (Fig. 1).

An 18F-fluorodeoxyglucose positron emission tomography-computed tomography scan (FDG PET-CT scan) was performed and showed abnormal metabolic activity in the spleen, and in multiple lymph nodes located in the cervical, mediastinal, hepatic, periaortic and iliac regions (Fig. 2).

A biopsy of the supraclavicular lymph node was performed. Direct smears showed over 100 acidfast bacilli per microscopic field, as illustrated in Fig. 3. A TB-specific PCR (GeneXpert MTB/RIF, Cepheid) performed on the same day gave a negative result for the Mtb complex.

Despite these early microbiological results, anti-TB treatment composed of rifabutin (300 mg day^{-1}), isoniazid (300 mg day^{-1}), ethambutol (800 mg day^{-1}) and pyrazinamide (1000 mg day^{-1}) began rapidly. Rifabutin was preferred to rifampicin to avoid drug-drug interactions with dolutegravir (Miller *et al.*, 2015). Five days later, the patient developed a biological hepatitis with liver enzymes five times over the upper normal limit. Pyrazinamide and isoniazid were stopped and liver function improved rapidly.

In the meantime, additional bacteriological investigations were performed. DNA was extracted from the lymph node using a commercial Fluorolyse kit (Hain Lifescience). The DNA strip assay, GenoType Mycobacterium CM (Hain Lifescience), confirmed the presence of the genus *Mycobacterium*, but did not provide a species identification. We performed DNA sequencing of the *rpoB* gene as described below.

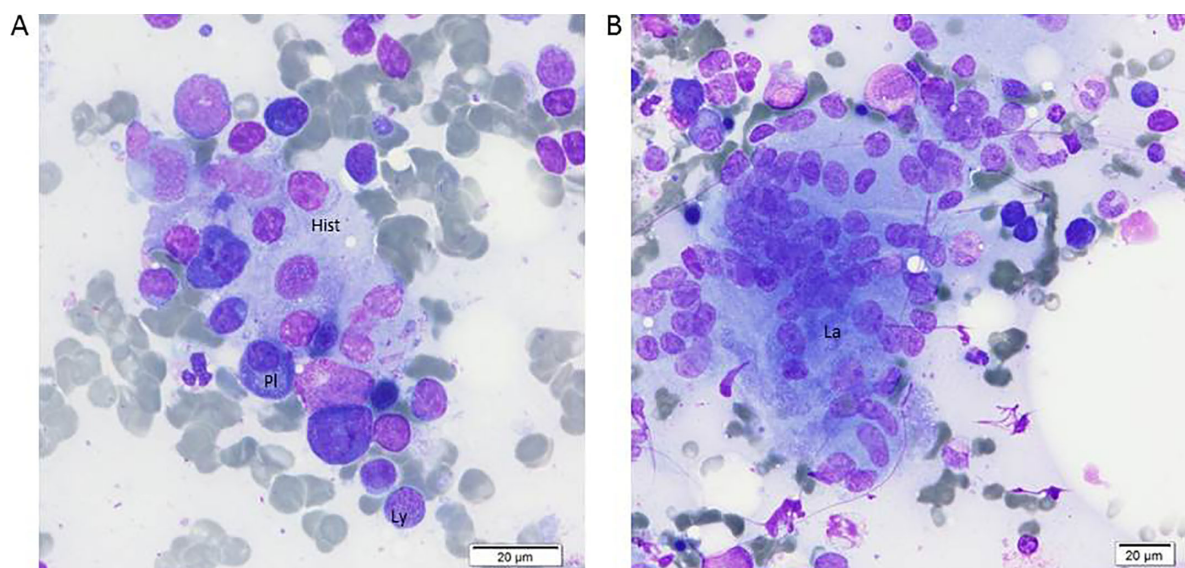


Fig. 1. Bone marrow. May–Grünwald Giemsa staining showing: (A) non-necrotizing epithelioid granuloma composed of epithelioid histiocytes surrounded by small lymphocytes (ly) and reactive plasmocytes (pl); (B) a multinucleated giant Langhans cell (La). Bars, 20 μm .

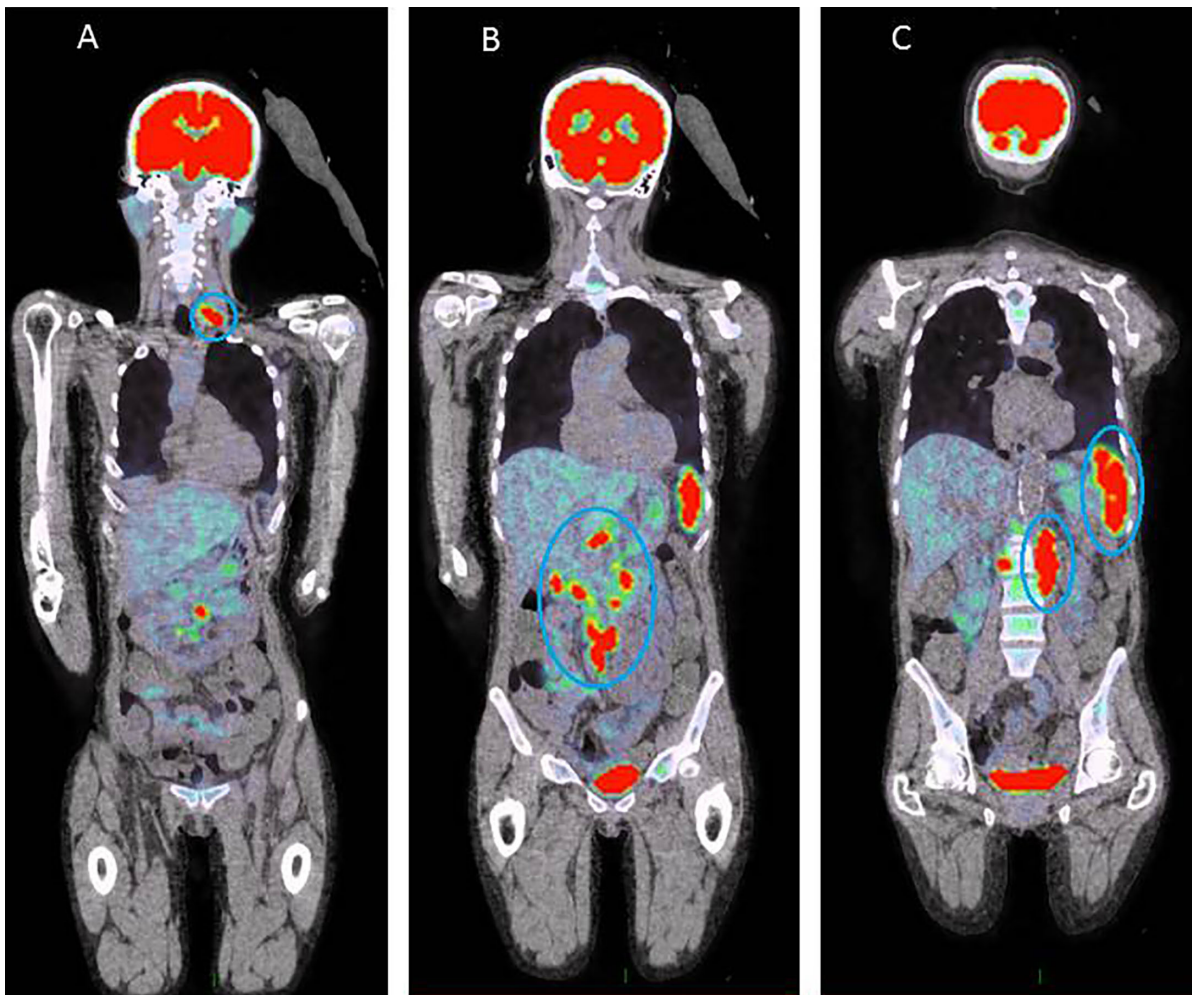


Fig. 2. FDG PET-CT scans indicating (areas circled in blue): (A) the supra-clavicular lymph node; (B) and (C) multiple hypermetabolic lymph nodes in the hepatic, periaortic and iliac regions. Note the high metabolic activity of the spleen.

As a first step, PCR amplification of the *rpoB* gene was carried out using the KOD Hot start master mix (Merck Millipore) with the primers, My9 and MycoR. These primers amplify a 800 bp region, presenting significant variations between mycobacterial species. Amplicons were purified with a QIAquick PCR purification kit (Qiagen) and diluted to 10 ng μl^{-1} .

The second sequencing step was performed on an ABI 3130 system (Life Technologies) using a BigDye Terminator v3.1 Cycle Sequencing and BigDye XTerminator Purification kit (Applied Biosystems, ref. 4376486) with primers My10, My11 and MycoF (Table 1).

A sequence of 643 bp was obtained, for which analysis was performed using a Smartgene Bacteria module (Smartgene), which had 100 % homology with *Mycobacterium genavense* (accession number HM022216).

This technique was applied to fresh samples and to samples which had been fixed in paraffin.

Based on these results, clarithromycin (500 mg b.i.d.) was added rapidly to the treatment as recommended in the ATS/IDSA guidelines (Griffith *et al.*, 2007) with good clinical evolution after one month.

Bone marrow and lymph node samples were cultured in an MGIT 960 instrument at 37 °C for ten weeks and in Lowenstein-Jensen media incubated at 30 °C, 35 °C and 42 °C for eight weeks. All these media remained sterile.

Discussion

Immune reconstitution inflammatory syndrome (IRIS) is a common complication of ART initiation among HIV patients, particularly in those with an advanced state of immunosuppression (CD4 count $<50 \mu\text{l}^{-1}$). It typically occurs as CD4 cell numbers increase and it is linked to the paradoxical worsening of pre-existing subclinical opportunistic infections due to pathogens such as TB and NTM, *Cryptococci* and *Cytomegalovirus* (Barber *et al.*, 2014; DeSimone *et al.*, 2000; French, 2012; Griffith *et al.*,

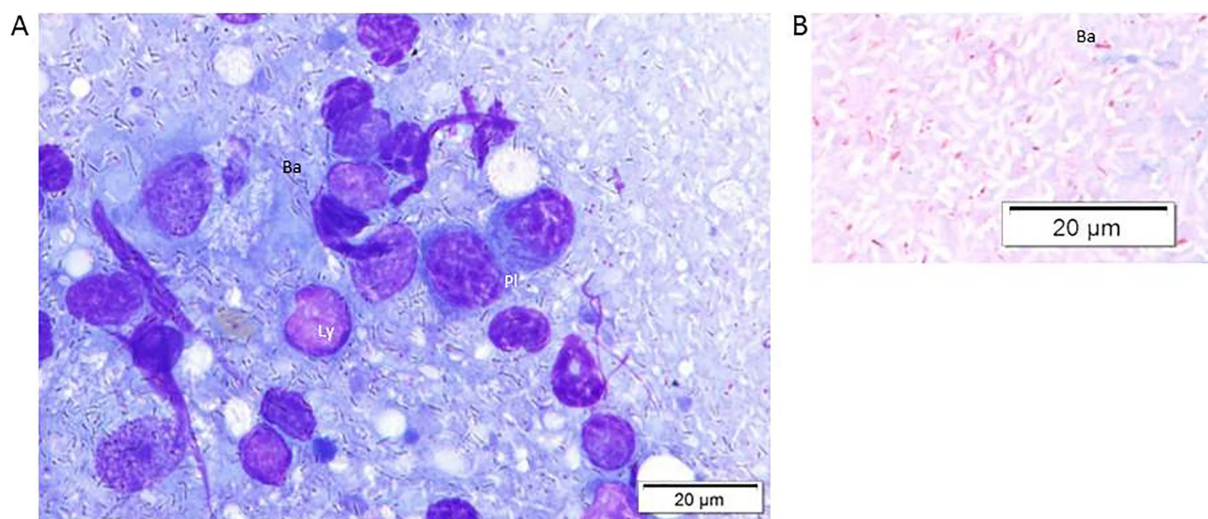


Fig. 3. Cervical lymph node. (A) May-Grünwald Giemsa staining showing poorly cohesive lymphocytes (Ly), reactive plasmacytes (Pl) and irregularly stained mycobacteria (Ba). (B) Ziehl-Neelsen staining showing numerous acid-fast bacilli (Ba) coloured in pink. Bars, 20 µm.

Table 1. Primers used for the amplification of the *rpoB* 'identification' region based on the *Mtb* H37Rv sequence (accession number AL123456.3)

Primer	Forward or reverse	Sequence (5'- 3')	Location	Source
My 9	F	ATCGGCGCCGAGGTCCGCGAC	2356–2376	This paper
My 10	F	AAGGTGCCSCACGGTGAGTC	2494–2517	This paper
My 11	R	ATGTAGCCGACCGTSACCGGGT	3044–3065	This paper
MycF	F	GGCAAGGTCACCCGAAGGG	2392–2411	(Adékambi <i>et al.</i> , 2003)
MycR	R	AGCGGCTGCTGGGTGATCATC	3135–3152	(Adékambi <i>et al.</i> , 2003)

2007; Walker *et al.*, 2015). IRIS can be severe and require the rapid initiation of an effective treatment.

Although *Mtb* remains the most prevalent species, in our particular experience (low incidence of TB), NTM represent an emerging problem (up to 50 % of *Mycobacteria* identified in the lab are non-TB species). NTM can be particularly severe among immunosuppressed patients, requiring a rapid diagnosis and rapid initiation of treatment (Murthy *et al.*, 2015; van der Werf *et al.*, 2014). In the clinical case presented above, rapid identification of a species of NTM directly from a clinical sample permitted the definite exclusion of *Mtb* infection and the initiation of a treatment oriented towards *Mycobacterium genavense*.

Mycobacterium genavense is a clinically relevant pathogen among immunocompromised patients, causing disseminated disease with serious morbidity and mortality (Santos *et al.*, 2014). The prevalence of these infections may be underestimated due to the difficulties in recovering the bacteria from the usual culture media, such as MGIT

and LJ, used in clinical practice (Thomsen *et al.*, 1999). Reports suggest that *Mycobacterium genavense* may grow on these media after up to 90 days of incubation at 45 °C in liquid media with an acid pH and Mycobactin J as a supplement. These specific requirements are not implementable in routine practice.

Rapid and accurate diagnostic methods for this growing group of opportunistic pathogens should, therefore, be made widely accessible. Using the technique described above, we were able to perform rapid species identification directly from smear-positive clinical specimens.

This technique has since been implemented in our routine workflow for smear-positive samples with negative *Mtb* PCR.

Acknowledgements

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