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Case Report

# Detection of *Mycobacterium tuberculosis*-derived DNA in circulating cell-free DNA from a patient with disseminated infection using digital PCR

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ABSTRACT

*Mycobacterium tuberculosis* (MTB) can disseminate to extrapulmonary organs, particularly in severely immunosuppressed patients. Confirmation of active MTB infection is often difficult in subjects with a contraindication for invasive procedures. A case of disseminated MTB infection after hematopoietic stem cell transplantation is reported herein. Circulating cell-free DNA from the patient showed positive amplification of an MTB complex-specific sequence using a digital PCR technique. The MTB infection was confirmed by positive acid-fast staining and an approved quantitative PCR assay using liver tissue obtained at autopsy. The detection of MTB in circulating cell-free DNA using this technique may represent a less invasive diagnostic tool for pulmonary and extrapulmonary MTB infections.


## Introduction

Although *Mycobacterium tuberculosis* (MTB) primarily causes lung infections, it can disseminate to extrapulmonary organs, particularly in subjects with severe immunosuppressive states, such as those with an HIV infection or post organ transplantation. In the case of extrapulmonary tuberculosis, invasive procedures are usually required to obtain specimens from lesions. These procedures are often contraindicated due to comorbidities, such as coagulation disorders or thrombocytopenia. The sensitivity of alternative tests using immunological reactions, the tuberculin skin test, and interferon-gamma release assays is variable in the immunosuppressive state (Pai et al., 2014). Therefore, reliable, simple, and less invasive diagnostic tests are required for MTB infection.

The digital PCR (dPCR) technique, in which amplification reactions are performed in a large number of fractionated partitions, can be used to quantitate extremely low copy number targets. This technique has been reported as more sensitive than quantitative PCR (qPCR) in both pathogen detection and gene mutation detection in cancer cells. A previous study by the present research group has also shown that MTB complex-specific DNA sequences can be quantitated by dPCR using circulating cell-free DNA (cfDNA) from smear-positive pulmonary tuberculosis (PTB) patients (Ushio et al., 2016).

A case of disseminated MTB infection is reported herein. Acid-fast staining and quantitative PCR of sputum, blood, and urine specimens did not detect the presence of MTB. dPCR using circulating cell-free DNA of the case showed positive amplification of MTB complex-specific sequences. The disseminated MTB infection was confirmed in autopsy samples from the case. Thus, the detection of MTB-specific sequences in cfDNA using dPCR could be exploited as a less invasive diagnostic tool for pulmonary and extrapulmonary MTB infections.

## Case report

The patient was a 63-year-male in a severe immunosuppressed state after a second hematopoietic stem cell transplantation

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(HSCT) for acute myeloid leukemia (AML) following relapse due to the first graft failure. Soon after achieving the second HSCT engraftment he developed a persistent fever, with pancytopenia and an aberrant coagulation status. Culture examinations of sputum and blood for bacterial pathogens, qPCR tests for major opportunistic viruses such as cytomegalovirus, Epstein–Barr virus, herpes simplex virus, human herpesvirus 6, and varicella zoster virus, and the β-D-glucan test as a screening test for fungal infections were all negative.

The systemic administration of prednisolone and tacrolimus was started for suspected graft-versus-host disease, but was discontinued due to persisting fever and relapse of the AML. A whole body computed tomography scan for the persisting fever showed the presence of mediastinal lymphadenopathy, with an area of central necrosis and peripheral rim enhancement, and multiple granular shadows in the whole lung. The latter finding is typical of a disseminated Mycobacterium infection, especially MTB (Figure 1).

Acid-fast staining, COBAS TaqMan MTB and MAI tests (Roche Diagnostics), and mycobacterial culture using sputum, urine, and blood samples were all negative. The TSPOT.TB test (Oxford Immunotec) was also performed, with negative results. Before the initiation of anti-tuberculosis chemotherapy without confirmation of an MTB infection, dPCR using cfDNA of the patient showed positive amplification for an MTB-specific genomic DNA sequence (13.1 copies and 0 copies per 20-μl reaction mix for insertion sequence 6110 (IS*6110*) and gyrase subunit B (*gyrB*), respectively).

The procedures used in this method have been reported previously (Ushio et al., 2016). Briefly, cfDNA from 200 μl of plasma was employed in the dPCR assay. The assay was conducted using the QX200 system (BioRad) and primers and probes designed to detect MTB complex genomic DNA-specific sequences, IS*6110* and *gyrB* (**Supplementary Material**, Table S1).

Although anti-tuberculosis treatment was initiated, the patient died of multiple organ failure. Pathological examination of the liver, kidney, and lung tissues obtained at autopsy revealed multiple caseous granulomas containing acid-fast stain-positive bacilli (Figure 1). Tissue mycobacterial culture of the liver did not show Mycobacterium growth. However, a COBAS TaqMan MTB test with DNA purified from formalin-fixed paraffin-embedded caseous necrotic liver tissue, in which acid-fast bacillus were contained, showed positive amplification, confirming the diagnosis of disseminated MTB infection.

## Discussion

*Mycobacterium tuberculosis* infection is a major life-threatening infectious disease worldwide, especially in immunocompromised hosts. Around 10 million new active tuberculosis cases and more than one million deaths were reported in 2015 (WHO, 2016). The respiratory tract is the most common site of MTB infection in the immunocompetent patient; however, extrapulmonary and disseminated MTB infections are more likely to be seen in those with immunosuppressed conditions, such as patients with an HIV co-infection or conditions related to medications and/or procedures. In severely immunosuppressed cases, imaging findings frequently reveal atypical features even in PTB. For example, patients with PTB and an HIV infection are less likely to have cavitary lesions than those without an HIV infection, and sometimes have normal chest radiograph findings, leading to delays in both MTB diagnosis and treatment. Following the negative results for culture and qPCR examinations using respiratory, urinary, and blood specimens in the case presented herein, consideration was given to performing a liver or bone marrow biopsy; however, a biopsy could not be performed due to the patient's severe abnormal coagulation status and thrombocytopenia.

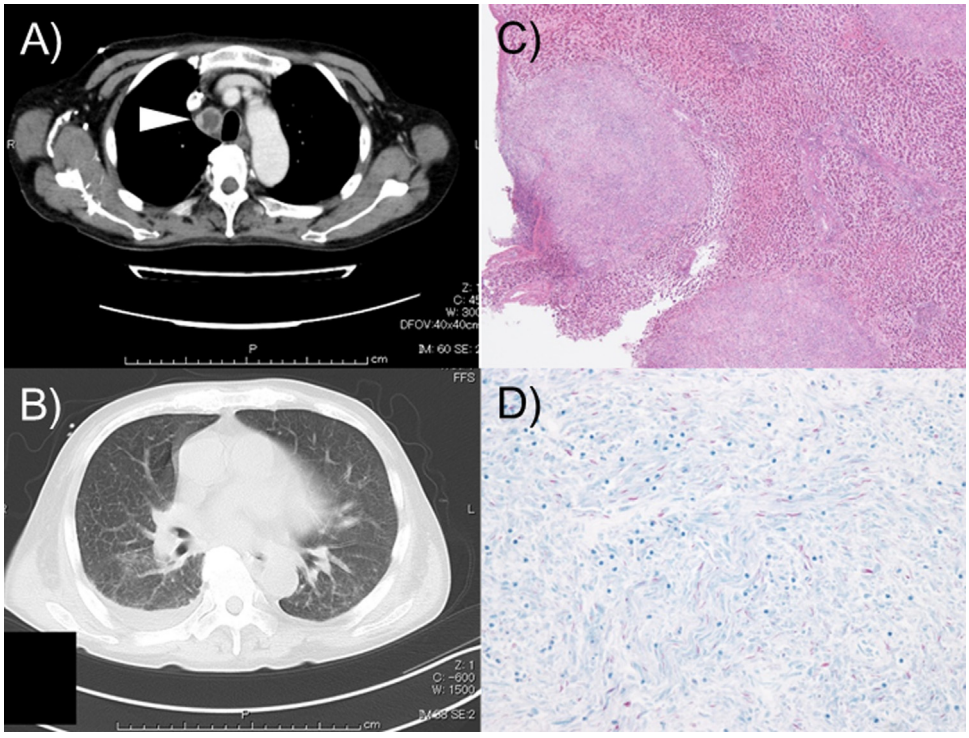


**Figure 1.** Computed tomography imaging findings before the initiation of anti-tuberculosis chemotherapy and histological and immunohisotochemical findings in the liver specimens obtained at autopsy. (A) Mediastinal lymphadenopathy with an area of central necrosis, and peripheral rim enhancement (arrow). (B) Randomly distributed multiple small nodules in both lungs. (C) Multiple caseous granulomas in the liver; hematoxylin and eosin staining (×200). (D) Positive immunohistochemical staining for acid-fast bacilli in the caseous granulomas from the liver (×400).

Several newly developed tests for MTB detection have recently become available. The sensitivity of the lateral flow urine lipoarabinomannan assay (LF-LAM), a urine-based test detecting a lipopolysaccharide present in the mycobacterial cell wall, is relatively low even in patients living with HIV (Shah et al., 2016). As for nucleic acid amplification, the Xpert MTB/RIF test has excellent sensitivity for MTB detection even in extrapulmonary specimens; however procedures are required to obtain specimens from the infection sites. Several studies assessing the utility of the Xpert MTB/RIF test using blood samples have reported inadequate sensitivity (Pohl et al., 2016). Therefore, an accurate MTB diagnostic test that uses easily accessible specimens, requiring less invasive procedures, is still needed.

The detection of MTB-derived DNA fragments in the blood using a PCR method was first reported in 1994. Recently, several reports have shown the dissemination of MTB across the body in the context of advanced immunosuppression (Lieberman et al., 2016). This finding might support the utility of blood as a specimen for diagnostic nucleotide amplification tests for MTB infection. With the dPCR technique, the sample is partitioned into a large number of fractions. Parallel end-point PCR reactions are then individually performed. These features enable the detection of a small number of targets in samples containing PCR inhibitors. The present researchers have already reported that dPCR for MTB detection using cfDNA showed better sensitivity than qPCR (Ushio et al., 2016). Another group has also reported the existence of an MTB-specific DNA sequence in circulating blood in both pulmonary and extrapulmonary tuberculosis patients (Yang et al., 2017). The possibility of dPCR using cfDNA for the detection of MTB in other than smear-positive PTB was shown in this case; however, the amplification reaction with one of the primer and probe sets was less than with the other. Refinements in procedures and primer/probe design are required to improve the sensitivity.

In conclusion, MTB infection is still a major life-threatening pathogen, especially in immunocompromised individuals. Extrapulmonary MTB infections are sometimes difficult to diagnose due to comorbidities. The detection of MTB-specific sequences with newly developed instruments for low copy number detection using cfDNA could be exploited as a less invasive diagnostic tool.

## Funding

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## Conflict of interest

None.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijid.2017.11.018.

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