Simultaneous detection of Mycobacterium bovis and M. tuberculosis in an apparently immunocompetent patient [Correspondence]

Article *in* The International Journal of Tuberculosis and Lung Disease · September 2013

DOI: 10.5588/ijtld.13.0127 · Source: PubMed

7 authors, including:

Gila Kahila Bar-Gal

Hebrew University of Jerusalem

73 PUBLICATIONS **1,150** CITATIONS

SEE PROFILE

Abedelmajeed Nasereddin

Hebrew University of Jerusalem

90 PUBLICATIONS **1,837** CITATIONS

SEE PROFILE

Kifaya Azmi

Al-Quds University

40 PUBLICATIONS **433** CITATIONS

SEE PROFILE

Charles Greenblatt

Hebrew University of Jerusalem

197 PUBLICATIONS **3,023** CITATIONS

SEE PROFILE

INT J TUBERC LUNG DIS 17(9):1242–1246

Correspondence

Simultaneous detection of *Mycobacterium bovis* and *M. tuberculosis* in an apparently immunocompetent patient

Mycobacterium tuberculosis remains the main cause of human tuberculosis (TB), with an unknown proportion of cases caused by *M. bovis*. Here we describe a case of pulmonary TB caused by mixed infection as studied from sequential sputum sampling and isolation of *M. tuberculosis* and *M. bovis* using a reverse dot blot (RDB) assay.

In December 2010, a 68-year-old human immunodeficiency virus negative male from the West Bank Palestinian territories was referred to the Palestinian Ministry of Health clinic with a history of cough, fever and dyspnoea for 6 months. The purified protein derivative skin test was negative; Ziehl Neelsen-stained sputum smear was positive.

Treatment with the Category I regimen was initiated. Mycobacterial growth was observed after 9 weeks of incubation of the sputum sample. Polymerase chain reaction (PCR) targeting the insertion sequence (IS) *6110* of the *M. tuberculosis* complex was found positive.[1] The isolate was further identified as *M. bovis* via multiplex-PCR as previously described.[2] The patient's travel history revealed a potential risk of exposure to TB, as he has been working and living for 12 years with workers from high TB prevalence countries where both *M. tuberculosis* and *M. bovis* are known to co-exist.[3]

In June 2011, a second isolate was identified as *M. tuberculosis* using the same methods as described above. At that point, treatment with the Category II regimen was initiated. For definitive identification of the causative pathogen, DNA was extracted directly from the decontaminated sediment of the smear-positive sputum samples taken at different dates. Three sputum samples along with the two isolates were tested by RDB assay, which included two amplification reactions targeting short amplicons of IS*6110* and the *oxy*R gene. Three probes were selected: an *M. tuberculosis* complex-specific probe derived from IS*6110*,[4] and two probes species-specific to *M. tuberculosis* and *M. bovis*, respectively, based on G/A transition of the *oxy*R gene.[5] All sputum samples gave three hybridisation signals indicative of mixed infection with the two mycobacterial pathogens (Figure).

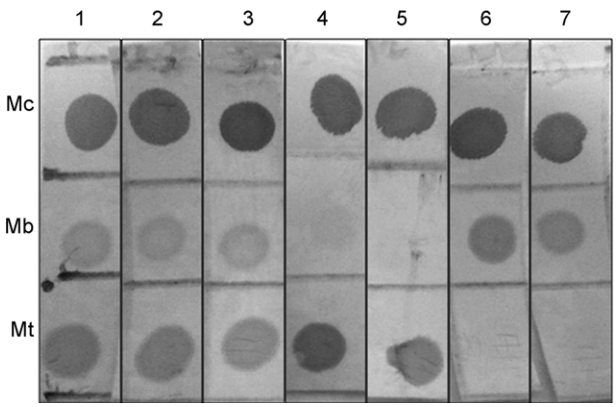

Figure Reverse dot blot hybridisation assay. Lanes 1–3: different sputum samples; Lane 4: positive control, H37Rv *M. tuberculosis* strain; Lane 5: clinical *M. tuberculosis* isolate 2; Lane 6: *M. bovis* BCG; Lane 7: clinical *M. bovis* isolate 1. Mc = *M. tuberculosis* complex-specific probe; Mb = *M. bovis*-specific probe; Mt = *M. tuberculosis* specific probes.

Despite treatment, the patient remained smear-positive, with progressive haemoptysis and weight loss (14 kg lost since diagnosis). In February 2012, the patient was transferred to a neighbouring country with suspected multidrug-resistant TB, but his condition deteriorated and he died before medical re-evaluation.

We assume that the patient was co-infected with both pathogens and that at a given time one pathogen became masked by the other during disease progression. The most likely scenario is that mixed infection was caused by reactivation of latent infection, reflecting his travel history. The delay in the final diagnosis of the causative pathogen resulted in inadequate treatment, particularly the use of pyrazinamide in the first 2 months of treatment and in the Category II regimen, as *M. bovis* is naturally resistant to pyrazinamide.

The RDB assay of sequential sputum samples confirmed the dual infection of both pathogens, as they were found to be present simultaneously in a single sample. Mixed infection with these two closely related pathogens was detected by isolation and identification of viable bacilli of both species from two different sputum samples, which may indicate that a culture of one sputum sample is not homogeneous and not representative of the total bacillary population in a patient.

Our report shows that the absence of specific identification of TB pathogens may have adverse consequences for patient management. The rare possibility of co-infection with these two pathogens in TB patients should be stressed to increase the awareness of potential multi-infection, particularly in geographical areas where both pathogens are known to co-exist.

Suheir Ereqat*†
Gila Kahila Bar-Gal†
Abedelmajeed Nasereddin*
Kifaya Azmi*
Issa Eshtaieh‡
Charles L. Greenblatt§
Ziad Abdeen*

*Al-Quds Nutrition and Health Research Institute, Faculty of Medicine
Al-Quds University, Abu-Deis
West Bank, Palestinian Authority
†The Koret School of Veterinary Medicine
The Hebrew University of Jerusalem
Rehovot, Israel
‡Central Public Health Laboratory
Palestinian Ministry of Health
Ramallah, Palestinian Authority
§Department of Microbiology and Molecular Genetics
The Hebrew University–Hadassah Medical School
Jerusalem, Israel
e-mail: sereqat@med.alquds.edu
http://dx.doi.org/10.5588/ijtld.13.0127

References

1 Eisenach K D, Cave M D, Bates J H, Crawford J T. Polymerase chain reaction amplification of a repetitive DNA sequence specific for *Mycobacterium tuberculosis*. J Infect Dis 1990; 161: 977–981.
2 Bakshi C S, Shah D H, Verma R, Singh R K, Malik M. Rapid differentiation of *Mycobacterium bovis* and *Mycobacterium tuberculosis* based on a 12.7-kb fragment by a single tube multiplex-PCR. Vet Microbiol 2005; 109: 211–216.
3 Prasad H K, Singhal A, Mishra A, et al. Bovine tuberculosis in India: potential basis for zoonosis. Tuberculosis (Edinb) 2005; 85: 421–428.
4 Thacker T C, Harris B, Palmer M V, Waters W R. Improved specificity for detection of *Mycobacterium bovis* in fresh tissues using IS*6110* real-time PCR. BMC Vet Res 2011; 7: 50.
5 Stermann M, Bohrssen A, Diephaus C, Maass S, Bange F C. Polymorphic nucleotide within the promoter of nitrate reductase (NarGHJI) is specific for *Mycobacterium tuberculosis*. J Clin Microbiol 2003; 41: 3252–3259.

Clinical experience of using clofazimine to treat multidrug-resistant tuberculosis

Globally, the emergence of multidrug-resistant tuberculosis (MDR-TB) is threatening global health. Favorable clinical outcomes are restricted by limited drug availability, the high rate of drug toxicity and the long duration of treatment.

Clofazimine was developed by Barry et al. in the 1950s as an anti-tuberculosis drug.[1] In vitro and in vivo trials in mice have recently demonstrated good activity against drug-resistant mycobacterial strains.[2] Some studies have reported favourable clinical outcomes using clofazimine to treat MDR-TB;[3–5] however, its clinical efficacy and safety remain to be determined. Here, we report our experience of using clofazimine in the treatment of MDR-TB.

Between February 2005 and December 2011, 32 patients were diagnosed with MDR-TB and treated with clofazimine-containing treatment regimens. All isolates were resistant to ofloxacin, and 11 patients (34.4%) had extensively drug-resistant TB. The daily dosage of clofazimine was 150 mg. Treatment success was defined as both cure and treatment completion. Of the 32 patients, 17 (53.1%) were men and the median age was 38 years (range 20–70). The identified isolates were resistant to a median of 9 drugs (range 4–13). Clofazimine was added to the treatment regimens for a median duration of 7 months (range 1–22). A median of four anti-tuberculosis drugs (range 2–7), excluding clofazimine, were administered over this period. Linezolid was administered to 15 patients, and 4 underwent surgical resection during the study period.

Clofazimine was discontinued in 11 patients due to stock-outs and in another 9 patients due to adverse events. Culture conversion was achieved in 17 of the 32 patients (53.1%). The median period from clofazimine treatment to culture conversion was 14 weeks (range 2–21 weeks). At data censure (15 February 2013), treatment outcomes were assessed in 31 patients, and 1 patient was still on a clofazimine-containing regimen after culture conversion. Treatment success was achieved in 15/31 patients (48.4%). Of the 15 successfully treated cases, 10 (66.7%) were treated with linezolid and 3 (20.0%) underwent surgical resection. Only 4 patients achieved treatment success without linezolid or surgical resection.

Skin discolouration developed in all patients. Four patients developed liver toxicity, and three developed gastrointestinal disturbance. Nine patients stopped receiving clofazimine due to adverse events, including skin discolouration ($n = 3$), hepatotoxicity ($n = 3$) and gastrointestinal disturbance ($n = 3$).

We conducted logistic regression analysis to identify factors for the success of treatment. According to the univariate analysis, male sex and linezolid are significant factors associated with treatment success. Multivariate analysis revealed that both male sex (odds ratio [OR] 16.67; 95% confidence interval [CI] = 1.15–242.45; $P = 0.039$) and linezolid use (OR 142.41; 95%CI = 2.07–9784.93; $P = 0.022$) are significant indicators of treatment success.

Our results show that clofazimine may be an effective treatment for MDR-TB; however, concurrent or subsequent use of linezolid may contribute to treatment success.

Jung-Wan Yoo
Jiwon Lyu
Sang Do Lee
Woo Sung Kim
Dong Soon Kim
Tae Sun Shim
Division of Pulmonary and Critical Care Medicine
University of Ulsan College of Medicine
Asan Medical Center
Seoul, South Korea
e-mail: shimts@amc.seoul.kr
http://dx.doi.org/10.5588/ijtld.13.0312