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One year experience using mycobacterial blood cultures to diagnose tuberculosis in patients with prolonged fever in Vietnam

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Abstract

Introduction—To evaluate the use of mycobacterial blood cultures (MBC) in diagnosing tuberculosis (TB) in patients with prolonged fever admitted to a Vietnamese referral hospital.

Results—MBCs from 94 patients (66% male; median age 33 years; 75% HIV positive) were evaluated: 14 were mycobacterium positive (all HIV positive), and MBC was the only positive specimen in 9 cases (41%). Three positive cases were identified as *Mycobacterium avium* and the remaining *M. tuberculosis* (one case could not be identified).

Conclusion—MBC can be a valuable additional method to diagnose TB, particularly in immunosuppressed HIV patients when sputum cannot be collected.

Keywords

tuberculosis; blood culture; HIV

Introduction

The Wolrd Health Organization estimated that 1.7 million people died of tuberculosis (TB) in 2009, including 1.3 million deaths in HIV-negative people and 0.4 million deaths in HIV-positive people. Vietnam is one of 22 countries with the highest burden of TB, where

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Author's contributions

NTND and HW wrote the manuscript and analyzed the data. HW, NVT, PVC and NVK designed the study. NTND, DTT and NTL participated in the collection of data. PH participated to analyze data. NTND and DTT participated labolatory testing. All authors have read and approved the final manuscript.

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approximately 32,000 people died from TB in 2009 and where multi-drug resistant TB (MDR-TB) is emerging [1]. It is important to have adequate TB diagnostics for both patient treatment and disease control. However, the diagnosis of tuberculosis can be challenging, especially in developing countries [2–4].

Several methods of TB diagnosis are commonly used worldwide, including: acid-fast bacilli (AFB) smear, culture of sputum on solid or in liquid medium, and molecular diagnostics [5,6]. HIV-infected patients with suspected pulmonary TB often lack a productive cough, thus sputum is difficult to collect [7]. Furthermore, the rate of sputum smear negativity is relatively high, ranging from 24% to 61% [8–10]. Patients with extra-pulmonary TB usually require invasive specimen collection procedures for TB diagnosis [11,12]. In such cases, blood culture for TB may help to confirm the diagnosis.

Mycobacterial blood cultures have shown varying diagnostic yields. One Brazilian study found mycobacteremia in 60% of 33 tuberculosis infected HIV patients and blood was the only positive specimen in 5 patients (15%) [13]. Another study in Spain showed that blood was the only positive sample in 33% of tested patients [14]. In this study we present the results of a one year observational study on the use of blood cultures to diagnose TB in patients with prolonged fever in northern Vietnam.

Methodology

Study population and specimen collection

This is a descriptive study on the routine clinical usage of mycobacterial blood cultures in patients admitted to the National Hospital for Tropical Diseases (NHTD) between September 2009 to September 2010 with prolonged fever of unknown origin. Patients diagnosed with prolonged fever where defined as those with more than two weeks of unexplained fever. Specimens were obtained at the discretion of the treating physician: bacterial and fungal blood cultures, sputum for AFB smear and TB PCR, cerebrospinal fluid (CSF), and aspirates (*e.g.* pleural fluid). All data were collected and stored anonymously for this descriptive study. This study was considered to be an evaluation of medical services by the Ethical Review Board of NHTD and the need for informed consent was therefore waived. In Vietnam the standard work up for TB is performing a sputum acid fast smear in suspect patients. Suspect patient are considered those with prolonged productive cough (>2 weeks). In case of HIV in the absence of productive cough, patients are treated empirically when TB is suspected. Induced sputum is generally not done as facilities to do this safely are not available.

Mycobacterial diagnostics

Sputum specimens were pre-processed using BBL MycoPrep Specimen Digestion/ Decontamination Kit (Becton Dickinson, Franklin Lakes, USA) according to manufacturer's instructions. The sediment was stained by Ziehl-Neelsen (ZN) to detect AFB and graded according to WHO recommendations by microscopy [15].

For mycobacterial blood cultures, 5 ml of blood was collected in Myco/F lytic (MFL) Bactec bottles (Becton Dickinson, USA) and incubated in the Bactec9050 instrument for 42

days. All positive MFL cultures were removed and AFB smear performed. MFL cultures that gave a positive signal within 42 days but did not contain AFB were returned into the instrument. MFL cultures positive with AFB were further tested for TB identification and drug resistance by GenoType MTBDRplus assay (HainLifesciences, Nehren, Germany). If MFL cultures were negative with GenoType MTBDRplus assay, *Mycobacterium avium* complex (MAC) PCR was performed, as described previously [16]. Routine TB PCR at our hospital is ordered at the discretion of the treating doctor and has IS6110 insertion sequence as target as described elsewhere [17].

Routine bacterial and fungal blood culture

Routine bacterial and fungal blood cultures were performed by Aerobic F Bactec (AF) bottles (Becton Dickinson, USA). In case of growth, bacteria or fungi were further identified using standard microbiological techniques.

Results

Patients with mycobacterial blood culture performed

During the one-year evaluation period, mycobacterial blood cultures were performed for 94 patients. Seventy patients (75%) were HIV positive, with relatively more men: 82%. All of the patients from which mycobacterial blood cultures were collected had signs suggestive of tuberculosis, including: prolonged fever of unknown origin, weight loss, cough, and fatigue. Fever lasted for a median of 27 days (IQR: 12-33.5) before blood for mycobacterial culture was collected. The characteristics of enrolled patients are summarized in Table 1.

From the 94 evaluated patients, AFB in any specimen was detected in 22 patients: blood cultures alone (n = 9), sputum smear alone (n = 5), PCR alone (n = 3), combination of blood culture and sputum smear (n = 4), combination of blood cultures and PCR (n = 1). A total of fourteen (15%) mycobacterial blood cultures were positive and all were from HIV-infected patients (Table 2). From these 14 cases, 13 strains were tested by the MTBDRplus assay (one specimen was accidentally discarded before testing). Ten patients were mycobacterial positive with this assay: 6 patients were susceptible with both rifampicin (RMP) and isoniazid (INH), 2 patients were resistant to both RMP and INH, 1 patient was resistant to RMP only, and 1 patient was resistant to INH only. Three cases that were negative with the MTBDRplus assay were all positive with *M. avium* by MAC PCR. The median time to positivity for mycobacterial blood cultures was 24.5 days (IQR: 20-27).

The CD4 counts for 50/70 HIV-infected patients were known and analyzed. Those who were mycobacterial blood culture positive never had CD4 counts exceeding 100 cells/mm^3. The mean CD4 count for mycobacterial blood culture positive patients was lower than for negative patients: 42.1 (95% CI: 21.3-62.9) versus 66.3 (95% CI: 40.3- 92.2) respectively, not significant. For 52/70 HIV-infected patients we had data on anti-retroviral therapy (ART). Generally those that were already on ART at admission had lower mycobacteremia rates as compared to those not on ART: 1/19 (5.3%) versus 9/33 (27.3%). The remaining four mycobacteremia cases we had no data on ART.

Sputum was collected from 61 patients for AFB smear. The other 33 patients did not submit sputum samples, generally due to non-productive cough. Nine (15%) of 61 patients were AFB positive. From 12 patients TB PCR was requested and performed for the following specimens: CSF (n = 9), sputum (n = 1), abdominal fluid (n = 1) and pleural fluid (n = 1). From these 12 patients, 4 patients were positive with tuberculosis (2 CSF, 1 abdominal fluid, 1 sputum). AF blood cultures bottles were collected from 87 patients to detect other bacterial and fungal pathogens. Of those, 19 patients were positive and *Penicillium marneffei* was the most commonly identified pathogen.

The median time of hospitalization of enrolled patients was 13 days (IQR: 8-22). Most patients were discharged before the result of MFL culture was available. Eight of the mycobacterial blood culture positive patients were transferred to a tuberculosis hospital for treatment. From the remaining six: four died, one went home and was readmitted to a TB hospital due to positive mycobacterial blood culture, and one was lost to follow up. One MAC positive case with follow-up data received MAC treatment when the result became known.

Discussion

In this study, a total of 22 out of 94 cases were diagnosed with a mycobacterial infection (any specimen) by all methods of which 14 cases (64 %) were blood culture positive. From the 14 blood cultures positive cases, 9 cases were positive by blood culture only. Hence, blood was the only positive specimen in 41% of mycobacteria infected cases. Our positive rate of blood cultures was much higher than positive blood cultures rates reported in another study conducted in Vietnam, Thailand and Cambodia [18]. This study performed mycobacterial blood cultures on HIV-infected outpatients, including those with no particular symptoms like fever, and therefore this population was less likely to have TB than our population [18].

AFB smear detected 9 cases of all 22 mycobacterial cases in our study. Other studies have also reported that in HIV patients with TB, a considerable proportion of sputum samples are negative for AFB [8,9]. One study compared AFB smears with mycobacterial culture and showed that AFB smears on three sputa failed to diagnose TB in 63% of HIV-infected patients [5]. However, another study showed that the best first step of TB diagnosis is still to perform AFB smear on at least 2 sputum specimens and if the smears are negative and TB is still suspected, sputum should be cultured [5]. Therefore it is essential also to culture sputum specimens in HIV-infected patients and not solely rely on AFB smear. This is a limitation of our study because sputum culture was generally not performed for various reasons, including: the patient cannot expectorate or was not requested by the doctor as sputum culture takes a long time and results are usually available after discharge. Blood cultures may also yield fungi, like *P. marneffei*, and therefore produce other relevant results for patient management.

In this study, HIV-infected patients with mycobacteremia are likely to have lower CD4 count than HIV-infected patients without mycobacteremia. *M.tuberculosis* was the most common cause of bloodstream infection (BSI) in HIV-infected cases in our study. This is consistent

with other studies reporting an association with low CD4 counts and BSI [18]. One study conducted in 3 countries including Vietnam observed that HIV-infected patients have a high likelihood of BSI, particularly if their CD4 count <100 cells/mm^3[18]. In our population, the majority (80%) of the patients had CD4 counts <100 cells/mm^3 as our study generally concerns HIV-infected patients who are admitted with opportunistic infections (AIDS defining illness). Mycobacteremia cases generally occurred in those not yet on ART. One mycobacteremia case occurred in a patients 2 months on ART but with remaining low CD4 counts, illustrating treatment failure for unknown reasons. Of the four cases that died in this study, one did not receive any TB treatment and the other three died one day after starting TB treatment. One lethal case was infected with MDR TB and one with a rifampicin resistant TB. Late installment of TB treatment was likely the cause of death in these fatal cases.

Mycobacteria were the most commonly found pathogens in the blood cultures in this study population, followed by *P. marneffei*. These results are consistent with a similar Asian study, where Myco/F lytic blood cultures were performed on HIV patients [18]. This study found that *M. tuberculosis* was the most frequent isolated pathogen in HIV patients, followed by *Cryptococcus* species and *P. marneffei*.

In resource constrained settings like Vietnam, mycobacterial blood culture is generally not performed. Hence, we believe our data on mycobacterial blood cultures can contribute to improve TB diagnosis in endemic TB countries in Asia, particularly in HIV-infected patients. Although many studies have different results regarding mycobacterial blood cultures, they could not negate the usefulness of blood cultures in helping to diagnose TB, especially when other specimens are difficult to collect or unavailable [18,19]. The yield of this method may be increased if more than one blood specimen is collected. However, costs and time to detection are high and therefore is generally not considered feasible.

Conclusion

Blood cultures for TB can be useful for TB diagnosis, particularly in immunosuppressed HIV patients when sputum or other specimens cannot be obtained. However, mycobacterial blood culture has some important limitations, including the long incubation time, costs, and sensitivity. mycobacterial blood cultures cannot replace sputum AFB smear and sputum culture, but is a valuable additional method to combine with other basic methods for better yield of TB testing. Methods to shorten time to detection of mycobacteria in the blood would improve the clinical utility.

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Table 1

Characteristics of 94 patients with prolonged fever of unknown origin.

Characteristics	All patients (n = 94)	Positive Mycobacterial Blood culture (n = 14)	Negative Mycobacterial Blood culture (n = 80)
Age, median (IQR)	33 (30-39)	31 (30-33)	34 (30-40)
Male sex	62 (66%)	11 (79%)	51 (64%)
Fever	94 (100%)	14 (100%)	80 (100%)
Cough	31 (33%)	4 (29%)	27 (34%)
Weight loss	33 (35%)	5 (36%)	28 (35%)
Fatigue	15 (16%)	5 (36%)	10 (13%)
HIV positive	70 (75%)	14 (100%)	56 (70%)
Hospitalization days Median (interquartile range)	13 (8-22)	16 (9-30)	12.5 (8-20)

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Table 2

Summary of 14 positive – TB blood cultures (MFL) from National Hospital of Tropical Diseases, September 2009-September 2010.

Patient	MFL Blood culture	TB PCR	AF blood culture	Sputum AFB smear	Time to positivity (days)*	Identificati on	RMP	INH	Outcome
1	Pos	ND	Pos	15	Neg	*M.avium*	ND	ND	Survived
2	Pos	Neg	Neg	Pos	21	MTB	R	S	Died
3	Pos	Neg	Neg	Neg	35	MTB	R	R	Survived
4	Pos	ND	Neg	Neg	33	MTB	S	S	Survived
5	Pos	Neg	Neg	ND	27	MTB	R	R	Died
6	Pos	Pos	Neg	ND	34	MTB	S	S	Died
7	Pos	ND	Neg	Neg	34	*M.avium*	ND	ND	Survived
8	Pos	Neg	Neg	Neg	20	MTB	S	S	Survived
9	Pos	ND	Neg	Neg	20	*M.avium*	ND	ND	Survived
10	Pos	ND	Neg	Pos	27	MTB	S	S	Survived
11	Pos	ND	Neg	Neg	20	ND	ND	ND	Survived
12	Pos	ND	Neg	Neg	24	MTB	S	S	Survived
13	Pos	ND	ND	ND	24	MTB	S	S	Died
14	Pos	ND	Neg	Neg	27	MTB	S	R	Unknown

*Time to positivity of TB blood cultures;; MTB: *M. tuberculosis;* ND: not done; R: resistant; S: susceptible