of other immunosuppressive agents has been found to reduce anti-
gen responses in quantiferon assays, including corticosteroids and
inhibitors of calcineurin and tumor necrosis factor-alpha.9,10 Care-
ful interpretation of borderline-positive quantiferon is important in
all children receiving immunosuppressive therapy due to their high
risk of developing TB disease if misinterpreted. Preferably, these
children should be screened before initiation of any immunosup-
pressive therapy.

Treating children for LTBI due to false positive results is not
without consequences as it includes (1) daily medicine admin-
istration for 3–9 months, which may be a challenge in children, (2)
outpatient consultations, (3) possible parental anxiety of a TB diag-
osis and (4) frequent adverse effects such as nausea and vomiting.
False positive results may also influence research in pediatric LTBI
by inclusion of non-truly infected children. No pediatric guidelines
are available for retesting children with previous false positive
results. Our population included primarily children without known
recent TB exposure, hence the results cannot be generalized to popula-
tions from high-endemic settings. We suggest that borderline-pos-
tive quantiferon results in children from low-prevalence settings
are repeated short- and long-term, in addition to evaluating TST,
to further explore the hypothesis of high risk of false positive results.

The limitations of this study include (1) the small number of
patients with borderline-positive quantiferon, (2) only one con-
trol quantiferon test in most children, (3) the potential influence
of methotrexate on the quantiferon result in 3 patients and (4) the
short follow-up of a median of 24 months. Therefore, this study is
not robust enough to make firm conclusions of false positive quan-
tiferon, only the presence of a high test variability in the border-
line range and, in some children, a high suspicion of false positive
results. Our population included primarily children with known
TB exposure, hence the results cannot be generalized to populat-
ions from high-endemic settings. We suggest that borderline-pos-
tive quantiferon results in children from low-prevalence settings
are repeated short- and long-term, in addition to evaluating TST,
to further explore the hypothesis of high risk of false positive results.

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MYCOBACTERIAL BLOOD CULTURE FOR
DIAGNOSIS OF TUBERCULOSIS IN VIETNAMESE
CHILDREN

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Abstract: Diagnosis of pediatric tuberculosis is notoriously difficult. We
investigated the additional yield of blood culture in hospitalized children in
Vietnam. Among 554 enrolled clinically suspected patients, an additional
6 cases were diagnosed, while the incremental cost per case was USD500.
Addition of blood culture is therefore not recommended for our total patient
population, but may be considered in specific groups.

Key Words: tuberculosis, children, diagnosis, mycobacterial blood culture

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were screened for meeting at least 2 inclusion criteria: unexplained fever (>2 weeks), unexplained cough (>2 weeks), chest radiograph suspicious for TB, evidence of malnutrition, failure to thrive/loss of weight, lymphadenopathy, suspected meningitis (>1 week), HIV infection and contact with TB cases. One blood sample (3–5 mL) was collected, and other diagnostic samples were collected as per standard of care. Clinical categories were determined retrospectively from the standardized case definition for intrathoracic TB in children published by Graham et al. Three categories were defined: confirmed, unconfirmed and unlikely TB. “Confirmed TB” was defined as microbiologically confirmed TB with at least one positive culture or WHO-endorsed nucleic acid amplification test (Xpert MTB/RIF) in any sample. “Confirmed TB” was defined as meeting at least 2 of the following criteria: defined signs or symptoms suggestive of TB; chest radiograph consistent with TB; close TB contact; or immunologic evidence of TB; and positive response to treatment. “Unlikely TB” was defined as not meeting criteria for confirmed and unconfirmed TB. Disseminated TB was defined as: (1) isolation of MTB from blood or ≥2 noncontiguous organs or (2) isolation of MTB from any organ with miliary pattern on the chest radiograph.

Blood was collected in BACTEC Myco/F Lytic culture bottles and incubated in the Bactec 9050 (Becton Dickinson, Sparks, MD) for 42 days following the manufacturer’s protocol. The added volume was not checked. Positive bottles were checked by Ziehl–Neelsen smear and susceptibility testing for isoniazid and rifampicin (GenoType MTBDRplus assay, Hain Lifescience, Roche Diagnostics, Switzerland).

**TABLE 1.** Characteristics of 16 Patients With Mycobacteremia

| Patient ID | Sex | Age (mo) | HIV status | Cough | Fever | Poor nutritional status† | Lymphadenopathy | Meningitis‡ | TB suspected chest radiograph | Clinical diagnosis | TB Treatment‡ | Outcome§ | MTB identified by routine culture |
|------------|-----|----------|------------|-------|-------|--------------------------|----------------|-------------|--------------------------------|------------------|--------------|----------|----------------------------------|
| 20-043     | F   | 7        | ND         | −     | +     | +                        | −              | +           | +                              | TBM              | +            | Withdraw + |                               |
| 20-065     | F   | 2        | ND         | +     | +     | +                        | −              | +           | +                              | Miliary TB       | +            | Withdraw + |                               |
| 20-097     | F   | 144      | ND         | −     | +     | −                        | −              | +           | NA                             | TBM              | +            | Survived + |                               |
| 20-106     | F   | 15       | −         | +     | +     | +                        | −              | +           | +                              | Miliary TB       | +            | Withdraw + |                               |
| 22-017     | F   | 9        | −         | +     | +     | +                        | −              | +           | +                              | PTB              | −            | Withdraw + |                               |
| 22-046     | M   | 84       | +         | +     | +     | +                        | −              | +           | +                              | PTB              | +            | Survived + |                               |
| 22-066     | M   | 2        | −         | +     | +     | +                        | −              | +           | +                              | Miliary TB       | +            | Survived + |                               |
| 22-084     | F   | 5        | −         | +     | +     | +                        | NA             | NA          | NA                             | +                | Survived + | +          | Miliary TB                     |
| 22-242     | M   | 13       | −         | +     | +     | +                        | +              | +           | +                              | Miliary TB       | +            | Survived + |                               |
| 22-327     | F   | 102      | ND        | +     | +     | +                        | +              | +           | +                              | Miliary TB       | +            | Survived + |                               |
| 20-018     | F   | 77       | +         | +     | +     | +                        | −              | −           | −                              | Bacteremia/HIV   | −            | +          | Miliary TB                     |
| 20-029     | M   | 1        | +         | −     | −     | −                        | −              | +           | +                              | Meningitis       | −            | +          | Survived + Meningitis           |
| 22-053     | M   | 4        | ND        | +     | +     | +                        | −              | +           | +                              | PTB              | −            | Survived + |                               |
| 22-072     | M   | 82       | −         | +     | +     | +                        | −              | +           | +                              | PTB              | −            | Survived + |                               |
| 22-101     | F   | 48       | −         | +     | +     | +                        | −              | +           | +                              | Miliary TB       | −            | Survived + |                               |
| 22-133     | M   | 13       | ND        | +     | +     | +                        | NA             | NA          | −                              | Pneumonia        | −            | +          | Survived + Pneumonia            |

† Poor nutritional status including one of the following status: malnutrition, weight loss, or fail to thrive.
‡ Symptoms of meningitis.
§ Receiving TB treatment during hospitalization.
¶ Patients whom their family decide to withdraw treatment due to the patient’s severe condition were defined as “withdrew.”
"Cases having negative routine culture.
ND, not done; NA, not available; M, male; F, female; PTB, pulmonary tuberculosis; +/−, present/absent.
Nehren, Germany). All samples, except cerebrospinal fluid, were decontaminated using the NaOH protocol. Pellets were split for microscopy, microscopic observation drug susceptibility assay, and liquid culture (MGIT). If insufficient volume was available, repeated sampling was attempted. All children’s parents/guardians gave written informed consent. The study protocol was approved by the Vietnam National Children Hospital Institutional Review Board (IRB-2010) and the Oxford Tropical Research Ethics Committee (13-10).

Two separate definitions of gold standard for pediatric TB: “confirmed TB gold standard” and “clinical gold standard” were used. The “confirmed TB gold standard” usually overestimates sensitivity and underestimates specificity of an evaluated test due to lack of sensitivity and this is the other way around with the “clinical gold standard” that lacks in specificity. We compared demographic and clinical characteristics of patients among diagnosed categories of TB using the Fisher's exact test for categorical variables and the Kruskal Wallis test for continuous variables. All analyses were done using SPSS version 21 (IBM Inc, Armonk, NY). Two sided P-values ≤ 0.05 were regarded as statistically significant.

Our study population represents the diversity of pediatric TB in 2 large referral hospitals in an endemic setting, where approximately half of children have extrapulmonary TB.5-7 Five hundred forty-four children, among whom 11 (11/554, 2%) were receiving treatment <1 week, were enrolled. 92 children (16.6%, n = 92/554) were classified as “confirmed TB,” 345 (62.3%, n = 345/554) as “unconfirmed TB” and 73 (12.3% n = 73/554) as “unlikely TB.” Forty-four children from whom no routine samples were collected, were classified separately and were excluded from the general analysis (Fig. 1). One thousand twenty-eight routine samples were collected from 510 patients: sputum (n = 81), gastric aspirates (n = 831) and others (n = 116). One sample was taken from 125 patients, 2 from 263 and 3–4 from 122.

Children’s baseline characteristics are shown in Table, Supplemental Digital Content 1, http://links.lww.com/INF/D600. The median age was 29.9 months (IQR: 10.9–85.2). Evidence of past BCG vaccination was observed or reported from 92.2% (n = 470/510); 25.3% (n = 129/510) had a TB contact within the previous year, 12% (n = 15/129) of whom were household members. The clinical findings included fever (79.4%), persistent cough (82.4%), weight loss (44.2%), failure to thrive (54.9%), lymphadenopathy (11.5%) and chest radiograph consistent with TB (61.7%) (Table, Supplemental Digital Content 2, http://links.lww.com/INF/D601). The median history of illness was 30 days (IQR 6.45–90).

Thirty-two among 554 children had a positive blood culture, among whom 22 were confirmed MTB (2.9%) (Table, Supplemental Digital Content 3, http://links.lww.com/INF/D602). Fifteen of these 16 children had other samples collected (Table, Supplemental Digital Content 4, http://links.lww.com/INF/D603), two were AFB smear positive and 5 had negative routine cultures (Fig. 1). Thus, mycobacterial blood culture increased the number of confirmed cases from 92 to 97. The 5 additional cases were susceptible to rifampicin and isoniazid. Among 28 immunocompetent patients, as determined by clinical diagnosis, the yield of MTB blood culture was lower than that of routine liquid culture (6/28, 21.4% vs. 21/28, 75%). Among the 15 mycobacteremia cases with routine samples available, the median time to detection was 26 days (IQR: 13.0–31.0) for mycobacterial blood culture and 14 days (IQR: 8.8–16.0) for routine culture. The demographic and clinical characteristics of patients with MTB isolated from blood are summarized in Table 1. Among 16 cases with MTB bacteremia, 10 presented with disseminated TB (3 with tuberculous meningitis and 7 with miliary TB). Two patients were HIV infected. In the bivariate analysis, HIV infection did not show any association with mycobacteremia (22.2% vs. 5.6%, P = 0.105).

Unfavorable outcome (death or pulmonary discharge) was more frequent in patients with mycobacteremia (26.7% vs. 5.5%, P = 0.01) (Table, Supplemental Digital Content 5, http://links.lww.com/INF/D604). One of 16 cases with mycobacteremia had no routine cultures taken. Among the other 5 cases with mycobacterial blood culture as the sole source of MTB, there were 2 cases who already received TB treatment with a final clinical diagnosis of miliary TB and the culture became positive. The remaining 3 cases had not received TB treatment at the time of discharge before the positive MTB growth in the blood sample was released.

The low culture confirmation rate (28.4%, 98/345) was similar to other studies in children.4,4 In a recent study in a TB hospital in southern Vietnam, there were also ~25% culture-confirmed cases.8 Adding blood culture to routine diagnostics only marginally increased the yield of TB confirmation and the added yield was small compared with the substantial additional cost: the costs of MycoF/ Lytic bottles alone per additional case detected was 525 USD.

Blood specimens are considered an attractive sample for diagnosis of pediatric TB because of the higher frequency of disseminated disease. However, here, only 21.4% cases were detected using blood culture when compared with 75% from respiratory samples using routine culture. An explanation may be that the mycobacteremia is transient and occurs before presentation at the hospital and blood collection, or that the mycobacteremia only occurs in a minority of children or that volume of blood collected for mycobacterial culture was not optimal.

In previous studies in adults,7-8 blood culture positivity for mycobacteria was more frequently found in HIV-infected patients but this difference was not statistically significant in our study population (2/15 in HIV positive vs. 5.6% in HIV negative (11/222), P = 0.105). This could be explained by the fact that not all children were tested and the number of children with confirmed HIV infection was small (n = 13/223 tests).

The time to positivity of mycobacterial blood culture in children with suspected TB in our study was long [median turnaround time (TAT): 25 days], similar to studies in adults.4,8 Recently, it was found that detection of MTB in blood using GeneXpert MTB/RIF could produce results within a day. The sensitivity of GeneXpert MTB/RIF was found to be similar to that of mycobacterial blood culture in a study of 104 HIV infected adults,10 but the high volume of blood used here (20 mL) could be problematic in small children.

Despite several limitations, our study demonstrates the utility of mycobacterial blood culture for TB detection in children: mycobacterial blood culture is able to detect more TB cases, but contributes little to the overall diagnostic yield in children.

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