ORIGINAL ARTICLE

WILEY

Bacteremia tuberculosis among HIV-negative children in China

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Funding source
National Natural Science Foundation of China, Grant/Award Number: 81401739; Beijing Talent Training Funded Project, Grant/Award Number: 2018000021469G277

Received: 8 March 2022
Accepted: 21 June 2022

DOI: 10.1002/ped4.12342

ABSTRACT

Importance: Bacteremia tuberculosis (TB) is a severe form of extrapulmonary TB. Studies assessing bacteremia TB in children are limited, especially for HIV-negative children.

Objective: To explore the detailed clinical features of the bacteremia TB in children under 18 years of age.

Methods: We reviewed the clinical records of the patients retrospectively and collected the strains isolated from their blood cultures. We used mycobacterial interspersed repetitive-unit-variable-number tandem-repeat (MIRU-VNTR) to characterize the bacterial genotypes and alamarBlue to determine their drug susceptibility profiles. Polymerase chain reactions and DNA sequencing were used to identify drug-resistant mutations.

Results: There were 13 pediatric bacteremia TB patients, 10 of whom were diagnosed with Bacillus Calmette–Guérin (BCG) bacteremia TB. Thirteen patients aged from 0.30 to 11.58 years were enrolled, of whom 76.92% were boys. All had fevers before hospitalization, and 76.92% had respiratory symptoms. All had received BCG vaccinations, and 46.15% had adverse post-vaccination reactions. Compared with *Mycobacterium tuberculosis*, BCG bacteremia was more likely to appear in younger children. Patients with BCG bacteremia had primary immunodeficiency diseases, and lower CD4, IgA, and IgE levels.

Interpretation: Bacteremia TB was rapidly fatal in a large proportion of the immunodeficient children. Because classic findings may not be diagnostically specific, a high level of clinical suspicion is required, especially for patients with certain types of immunosuppression. Studies are needed to develop rapid diagnostic tests and to determine the value of empirical therapy in childhood bacteremia TB.

KEYWORDS

Bacteremia tuberculosis, HIV negative, Children, China

INTRODUCTION

Tuberculosis (TB), caused by the *Mycobacterium tuberculosis* (MTB) complex, is the leading infectious cause of death worldwide.[1] It is a major global public health problem, and about one-third of the world's population is infected by MTB, predominantly in developing countries.[2] In 2017, about 10.0 million people developed TB disease, and the estimated number of deaths caused by TB was 1.6 million.[2]

While the lung is the organ primarily affected in TB patients, MTB infection can also affect other organs through the lymphohematogenous spread.[3] Typical extrapulmonary disease locations include lymph nodes, pleura, the genitourinary tract, bones and joints, meninges, peritoneum, and pericardium.[4,5] Extrapulmonary involvement coexists in 20%–30% of the pulmonary TB cases, mainly due to lymphatic and, in some cases, hematogenous spread. Children under 5 years of age are at a high risk of disseminated TB disease, with the youngest children suffering from the highest risk of dissemination.[6]

MTB complex bacteremia was first documented by Faber in 1914.[7] Clough[8] reported five patients with MTB complex bacteremia in 1917 and Shapira[9] reported another seven patients in 1932. Isolation of MTB from blood cultures has been reported since the early 20th century from patients with miliary tuberculosis.[10] During the past two decades, the diagnosis of MTB complex bacteremia has been made with increasing frequency.[11] This appears to be due to increased awareness following the human immunodeficiency virus (HIV) epidemic and the availability of new culture systems for the rapid detection of MTB. Despite this, MTB complex bacteremia is reported predominantly in adults, even in high HIV prevalence areas, and is well described in acquired immunodeficiency syndrome (AIDS) cases but not in HIV-negative patients.[12–14] In HIV-infected adults living in high tuberculosis-burden settings, MTB complex bacteremia is one of the most common causes of bloodstream infections with prevalence estimates as high as 20% and case-fatality ratios near 50%.[15] However, studies assessing MTB complex bacteremia in children, especially in HIV-negative children are limited and the detailed clinical features of MTB complex bacteremia in children have not been described.

Given the difficulty in establishing the diagnosis of MTB complex bacteremia in many settings and the need for prompt initiation of antituberculosis therapy to reduce mortality, we present 13 HIV-negative MTB complex bacteremia patients with positive results of blood culture in one of the largest children's hospital in China. We sought

to determine detailed clinical features of children under 18 years of age with MTB bacteremia.

METHODS

Ethical approval

This study was approved by the Institutional Review Board of Beijing Children's Hospital, Capital Medical University (approval no. 2014-47). The study was exempt from requirements to obtain informed consent from the patients.

Patient population

Patients with a positive culture of blood for mycobacterium were enrolled between January, 2016, and December, 2018. All the patients were under 18 years of age and treated in inpatient settings at Beijing Children's Hospital.

Data collection

We reviewed the clinical records of the patients retrospectively and collected the data, including demographic characteristics, clinical features, hematologic and biochemical tests, imaging diagnosis, microbiologic investigations, anti-TB treatment and outcomes, genotyping, and molecular characteristics.

Sample collection

All specimens were collected for diagnostic purposes. The strains isolated from blood were sent to Beijing Chest Hospital for the conventional drug susceptibility test and genotyping.

Drug susceptibility test

To determine the minimal inhibitory concentrations (MICs) of the strain isolates in this study, the alamarblue assay was performed as described previously.[16] Briefly, bacterial clones were scraped from the 4-week-old cultures grown in Lowenstein–Jensen medium. After vigorous mixing on a vortex mixer for 1 min, the suspension was diluted with 0.9% sodium chloride (normal saline) to 1.0 McFarland turbidity. To further prepare the inoculum, the 1.0 McFarland cell suspension was diluted at 1:20 with Middlebrook 7H9 broth containing 10% OADC. Then, 100 μL of this inoculum was inoculated into each well of a 96-well plate, and 100 μL of 7H9 broth, with a continuous concentration of each drug, was added to each well. After 8 days of incubation at 37°C, 70 μL of alamarBlue solution was added to each well for the assay. After an additional 24 h of 37°C incubation, the color change was used to assess bacterial growth, and MIC was defined as the lowest drug concentration of color from blue to pink. All experimental results were performed in triplicate. MIC breakpoint concentrations were defined as 1.0 $mg{\cdot}L^{-1}$ for rifampicin (RIF), 0.1 $mg{\cdot}L^{-1}$ for isoniazid (INH), 1.5 $mg{\cdot}L^{-1}$ for levofloxacin (LFX), 1.0 $mg{\cdot}L^{-1}$ for amikacin (AMK), 2.0 $mg{\cdot}L^{-1}$ for moxacin (MOX), 4.0 $mg{\cdot}L^{-1}$ for aminosalicylic acid (PAS), and 2.5 $mg{\cdot}L^{-1}$ for capreomycin (CPM).

Genotyping

Genomic DNA was extracted from 4-week-old cultures grown in Lowenstein–Jensen medium as previously reported.[17] Bacterial colonies were scraped from the surface of the medium and transferred to a 1.5 mL centrifuge tube containing 500 μL of Tris-EDTA (TE) buffer. After inactivation in a 100°C water bath for 30 min, polymerase chain reaction (PCR) amplification was carried out using the supernatant containing the DNA template. All the isolates were genotyped using the classical 15-loci mycobacterial interspersed repetitive-unit-variable-number tandem-repeat (MIRU-VNTR) method described by Supply et al.[18] The PCR products were analyzed with 1.5% agarose electrophoresis at 120 V for 45 min using a 50 bp DNA ladder (Takara, Japan) as a size marker. Amplicon sizes were estimated using Quantity One software (Bio-Rad, Hercules, CA, United States). The corresponding number at each locus was calculated according to the repeat and flank length.

PCR amplification and DNA sequencing

Fragments of rpoB, katG, the inhA promoter, and intergenic region oxyR-ahpC were amplified by PCR. The primer pairs were synthesized as previously reported.[19] After purification with the QIAquick PCR purification kit (Qiagen, Hilden, Germany), the DNA amplicons were sent to Rui Biotech Company (Beijing, China) for sequencing. ClustalW software was used to analyze the final nucleotide sequences in comparison with the H37Rv wild-type genome sequence.

The resulting sequences were compared with the homologous sequences of the reference *M. tuberculosis* H37Rv strains using BLASTn optimized for MegaBLAST on the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov/BLAST).

Statistical analysis

The Fisher exact test and Mann–Whitney *U*-test were used to compare the difference between Bacillus Calmette–Guérin (BCG) bacteremia and MTB bacteremia. Two-sided *P* values of < 0.05 were considered statistically significant. The statistical analysis was performed in SPSS 15.0 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Demographic characteristics

From January 2016 and December 2018, there were 21 patients with culture-positive results from blood samples. Due to strain loss, contamination, and incomplete clinical information, 13 patients were finally enrolled in our study. Of the 13 patients aged from 0.30 to 11.58 years (median: 0.39 years), 10 (76.92%) were boys and seven (83.85%) were from the rural area of China. All the patients were from provinces located in north China, including Heibei (3, 23.08%), Shandong (3, 23.08%), Beijing (2, 15.38%), Inner Mongolia (2, 15.38%), Jilin (1, 7.69%), Liaoning (1, 7.69%), and Shanxi (1, 7.69%). The parents of patients who lived in rural area were all farmers, while those who lived in the urban area were office workers, teachers, farmers, or unemployed (Table S1 in the supporting material).

Clinical features

All of the patients had a fever during the prehospital period, 10 (76.92%) had respiratory symptoms (seven with cough and seven with dyspnea), four (69.23%) had a rash, five (61.54%) had lymphadenectasis, and one (7.69%) had neurological symptoms. Only one (16.67%) patient had a history of TB contact. All patients had received a BCG vaccination and six (46.15%) of them had adverse reactions after BCG vaccination, including scar ulceration (4, 66.67%), lymph nodes in the left axilla (3, 50.00%), and fever after the vaccination (1, 16.67%) (Table S2).

Underlying sources of immunosuppression are also summarized in Table S2. Primary immunodeficiency diseases[20] and hematologic diseases[21] were present in nine patients (69.23%) and three patients (23.08%), respectively, and one patient (7.69%) had no apparent immune disorder.

Hematologic and biochemical tests

The results of hematologic and biochemical tests are summarized in Table S3. Patients with MTB complex bacteremia had several abnormal test results. The median values of a neutrophil count (67.50×10^9 L^{-1}), aspartate aminotransferase (62.80 $U{\cdot}L^{-1}$), and B lymphocyte (48.40%) in TB bacteremia patients were higher than normal ranges. The median values of hemoglobin concentration, albumin, creatinine, uric acid, CD3, CD4, CD8, natural killer (NK) cells, IgA, IgM and IgE were lower than their normal ranges in MTB complex bacteremia patients. Total white blood cell count, platelet count, C-reactive protein, alanine aminotransferase, total bilirubin, urea, and IgG were within normal ranges.

Imaging diagnosis and microbiologic investigations

Chest radiographs suggested miliary tuberculosis for two patients (15.38%), pneumonia for eight patients (61.54%), bronchitis for two patients (15.38%), pleural effusion for two patients (15.38%), and lung hernia for one patient (7.69%) (Table S4).

Seven patients had acid-fast bacillus (AFB) smear microscopy, of which five samples were sputum (one positive), one pleural fluid (none positive), and one gastric juice (one positive). Three patients had a tuberculin skin test (one positive) and nine patients had T-SPOT.TB test (three positive) (Table S4).

Anti-TB treatment and outcome

Of the six patients who received anti-TB treatment, four patients had the treatment immediately after TB clinical diagnosis,[22] and one patient took the treatment 17 days before TB diagnosis who were suspected to have TB on the basis of clinical symptoms. The condition of five patients improved when out of the hospital, whereas eight patients died who received the culture results 5.50 days after death on average. The median time between symptom onset and admission to the hospital was 15 days, and that between admission to the hospital and TB diagnosis was 21 days. The patients who took anti-TB treatment were significantly associated with a better prognosis (80.00% *vs.* 25.00%, $P < 0.05$). Of eight patients who died, just one patient received the prophylactic anti-TB treatment with isoniazid in the absence of a definitive TB etiological diagnosis (Table 1).

Genotyping and molecular characteristics

The 15-loci MIRU-VNTR revealed that 10 of the 13 patients had the same MIRU-VNTR pattern as BCG, while the MIRU-VNTR patterns of the other three patients (Patients 9, 10, and 12) were similar to H37Rv, the standard strain of MTB. Among three MTB strains, the isolates from patients 10 and 12 belonged to one cluster (Figure 1).

Based on drug susceptibility test results, two isolates (15.38%) were drug-resistant MTB. The isolate from Patient 9 was resistant to both INH and ethionamide (EMB), and the isolate from Patient 12 was resistant to EMB. The sequencing results confirmed that the isolates from Patient 9 and Patient 12 had mutations in drug-resistance related genes (Table S5).

Comparisons between BCG bacteremia and MTB bacteremia

We further compared the characteristics of BCG bacteremia and MTB bacteremia patients. Compared with

TABLE 1 Anti-TB treatment and outcomes for the 13 bacteremia TB patients

Patient	Anti-TB treatment	INH	RFP	PZA	SM	EMB	PTO	LZD	FQ	Outcome	Interval between symptom onset and hospital admission (Days)	Interval between hospital admission and TB diagnosis (Days)	Culture result turnaround Time (Days)	Interval between TB diagnosis and anti-TB treatment (Days)
1	No	×	×	×	×	×	×	×	×	Death	30	18	16 days after hospital discharge	/
2	No	×	×	×	×	×	×	×	×	Death	11	29	13 days after death	/
3	Yes	√	√	√	×	×	×	×	×	Condition improved	10	21	13 days after hospital discharge	17 days before TB diagnosis
4	No	×	×	×	×	×	×	√	×	Condition improved	60	21	15 days after hospital discharge	/
5	No	×	×	×	×	×	×	×	×	Death	7	28	23 days after death	/
6	Yes	√	√	×	×	×	×	×	×	Condition improved	90	29	19 days after hospital discharge	NA
7	No	×	×	×	×	×	×	√	×	Death	45	20	14 days after death	/
8	Yes	√	×	×	×	×	×	×	×	Death	60	35	9 days after death	0
9	Yes	×	√	√	×	√	×	√	√	Condition improved	1	57	53 days before hospital discharge	5
10	Yes	√	√	×	×	×	×	×	×	Death	19	6	2 days after death	1
11	No	×	×	×	×	×	×	×	×	Death	30	32	14 days after death	/
12	Yes	√	√	√	×	√	×	√	×	Condition improved	15	3	3 days before hospital discharge	0
13	No	×	×	×	×	×	×	√	×	Death	45	24	22 days after death	/

Abbreviations: EMB, ethionamide; FQ, fluoroquinolones; INH, isoniazide; LZD, linezolid; NA, not available; PTO, protionamide; PZA, pyrazinamide; RFP, rifampicin; SM, streptomycin; TB, tuberculosis.

MTB bacteremia, the BCG bacteremia was more likely to appear in the younger children (1.44 *vs.* 8.96 years, $P = 0.007$) and in children with primary immunodeficiency (90.00% *vs.* 0.00%, $P = 0.014$). A lower level of CD4 (7.65% *vs.* 39.60%, $P = 0.030$), IgA (0.24 *vs.* 1.30 $g{\cdot}L^{-1}$, $P = 0.030$) and IgE (11.98 *vs.* 32.53 $IU{\cdot}mL^{-1}$, $P = 0.030$) were observed in BCG bacteremia patients. In addition, the BCG bacteremia patients also had a higher level of aspartate aminotransferase (585.30 *vs.* 22.65 $U{\cdot}L^{-1}$, $P = 0.009$), and had a lower positive rate of T-SPOT.TB (100.00% *vs.* 0.00%, $P = 0.002$), were less likely to access anti-TB treatment (0.00% *vs.* 100.00%, $P = 0.035$), and had shorter hospital stays (22.00 *vs.* 71.00 days, $P = 0.028$) (Table 2).

DISCUSSION

Although young age is a risk factor for the most severe forms of TB, available evidence suggests that MTB complex bacteremia is uncommon among young children.[23] The rarity of MTB complex bacteremia identified in children could reflect a unique pathology of the disease in pediatric populations compared to adult populations.[24]

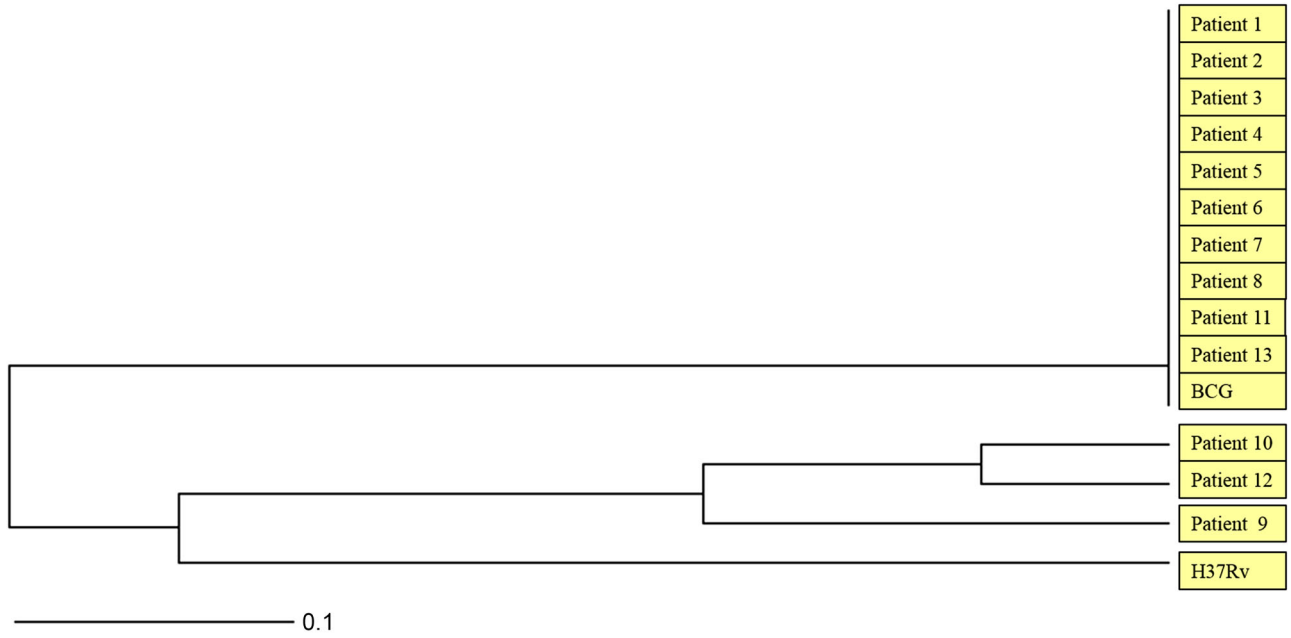

FIGURE 1 An upgma-tree based on 15-loci MIRU-VNTR of the 13 clinical isolates from the bacteremia tuberculosis patients. MIRU-VNTR, mycobacterial interspersed repetitive-unit-variable-number tandem-repeat; BCG, Bacillus Calmette–Guérin.

Our study revealed the detailed clinical and molecular characteristics of childhood MTB complex bacteremia in 13 patients. As far as we are aware, this is the first study of MTB complex bacteremia in the largest sample so far of HIV-negative children considering various characteristics, including demography, clinical features, hematologic and biochemical tests, imaging diagnosis, microbiologic investigations, treatment and outcome, genotyping and molecular characteristics. In our study, the patients with childhood MTB complex bacteremia had a very high fatality rate (61.54%, 8/13), which is consistent with previous reports.[25] The high case fatality rate of childhood MTB complex bacteremia underscores the importance of early diagnosis and early treatment of this disease.

First, our study showed that MTB complex bacteremia patients presented with undifferentiated fever and respiratory symptoms at admission. The clinical signs and derangements of basic hematologic and biochemical tests were not specific for the diagnosis of MTB complex bacteremia and most patients had no history of TB contact. Chest radiographs revealed that miliary shadowing occurred in only a minority (15.38%) of patients in our study, which emphasizes that this sign cannot be relied on to rule out the diagnosis of MTB complex bacteremia. For these reasons, the early diagnosis of childhood MTB complex bacteremia is very difficult. However, most of the patients had underlying sources of immunosuppression, such as primary immunodeficiency diseases and hematologic disorders, and abnormal values of immunity indexes, such as CD3, CD4, CD8, B lymphocyte, NK cell, IgA, IgM, and IgE. This underscores the need for clinicians to maintain a high degree of clinical suspicion for MTB complex bacteremia in caring for children with underlying sources of immunosuppression, particularly for children with abnormal values of immunity indexes.

Second, our research also emphasizes the problems associated with long turnaround times for culture results. Culture-confirmation of MTB complex bacteremia may not be available timeously when key clinical management decisions need to be made. It takes at least 4–6 weeks to obtain the results for mycobacterial culture, and the turnaround time is even longer in children with the characteristically paucibacillary disease. Recent data suggests that approximately 50% of HIV-infected adults with MTB complex bacteremia die within 30 days of their hospital presentation.[15] As shown in our study, except for two patients who took the anti-TB treatment according to the culture results on account of the long hospital stay, the remaining 11 patients all received the culture results after their hospital discharge or death. Moreover, AFB smear microscopy, the tuberculin skin test, and T-SPOT.TB did not contribute enough to the diagnosis of such patients in our study, either because the test was not performed or because the results were negative. The main reason may be that BCG bacteremia patients are absent from T-SPOT.TB antigens account for the vast majority of our study population. Ideally, molecule-based methods could be used for such diagnoses but they have not shown great promise in obtaining quicker results from blood samples due to low sensitivity, even with high volume extraction.[26] Given these

TABLE 2 Comparisons between BCG bacteremia and MTB bacteremia cases

Variable	BCG bacteremia	MTB bacteremia	*P*
Demographic characteristics			
Age	1.44 (0.30–2.58)	8.96 (6.33–11.58)	0.007
Gender (male)	8 (80.00)	2 (66.67)	1.000
From rural area	6 (60.00)	0 (0.00)	0.192
Clinical presentation at admission			
Fever	10 (100.00)	3 (100.00)	1.000
Rash	4 (40.00)	0 (0.00)	0.497
Lymphadenectasis	4 (40.00)	1 (33.33)	1.000
Neurological symptoms	1 (10.00)	0 (0.00)	1.000
Respiratory symptom	9 (90.00)	1 (33.33)	0.108
Adverse reaction after BCG vaccination	6 (60.00)	0 (0.00)	0.192
Source of immunosuppression			
Hematologic diseases	1 (10.00)	2 (66.67)	0.108
Primary immunodeficiency diseases	9 (90.00)	0 (0.00)	0.014
No apparent immune disorder	0 (0.00)	1 (33.33)	0.231
Hematologic and biochemical tests			
Blood routine			
Total WBC count, cells $\times 10^9$ L^{-1}	5.08 (2.94–7.21)	3.44 (3.08–3.79)	0.077
Neutrophil count, neutrophils $\times 10^9$ L^{-1}	37.90 (22.40–53.40)	72.80 (72.30–73.30)	1.000
Hemoglobin concentration, $g \cdot L^{-1}$	78.00 (87.00–89.00)	101.50 (100.00–103.00)	0.287
Platelet count, platelets $\times 10^9$ L^{-1}	103.00 (51.00–155.00)	244.00 (165.00–323.00)	0.287
C-reactive protein, $mg \cdot L^{-1}$	42.75 (13.00–72.50)	47.00 (8.00–86.00)	0.811
Hepatorenal function			
Albumin, $g \cdot L^{-1}$	28.50 (22.60–34.40)	29.30 (27.10–31.50)	0.727
Alanine aminotransferase, $U \cdot L^{-1}$	200.20 (30.90–369.90)	95.50 (72.80–118.20)	0.482
Aspartate aminotransferase, $U \cdot L^{-1}$	585.30 (62.80–1107.80)	22.65 (0.00–45.30)	0.009
Total bilirubin, $\mu mol \cdot L^{-1}$	16.95 (12.90–20.99)	9.00 (7.00–11.00)	0.112
Creatinine, $\mu mol \cdot L^{-1}$	17.40 (13.30–21.50)	27.80 (21.10–34.50)	0.077
Urea, $mmol \cdot L^{-1}$	2.99 (1.72–4.25)	3.3 (2.06–4.69)	0.161
Uric acid, $mmol \cdot L^{-1}$	220.05 (185.90–254.20)	184.00 (135.80–232.20)	0.811
Cell–mediated immunity			
CD3, %	62.55 (31.60–93.50)	77.25 (70.60–83.90)	0.573
CD4, %	7.65 (4.50–10.80)	39.60 (39.40–39.80)	0.030
CD8, %	51.90 (24.60–79.20)	26.55 (22.00–31.10)	0.273
B lymphocyte, %	33.00 (2.40–63.60)	10.40 (0.10–20.70)	0.182
NK cell, %	4.30 (5.20–5.40)	7.50 (3.40–11.60)	0.485
Humoral immunity			
IgA, $g \cdot L^{-1}$	0.24 (0.07–0.42)	1.30 (0.69–1.90)	0.030
IgG, $g \cdot L^{-1}$	6.40 (5.66–7.14)	7.07 (6.00–8.13)	0.758
IgM, $g \cdot L^{-1}$	0.30 (0.04–0.57)	0.60 (0.50–0.69)	0.073
IgE, $IU \cdot mL^{-1}$	11.98 (5.00–18.96)	32.53 (32.18–82.87)	0.030

(Continues)

TABLE 2 (Continued)

Variable	BCG bacteremia	MTB bacteremia	*P*
Microbiologic investigations			
AFB smear microscopy	1 (25.00)	1 (33.33)	1.000
Tuberculin skin test	0 (0.00)	1 (50.00)	1.000
T-SPOT	0 (0.00)	3 (100.00)	0.002
Anti-TB treatment and outcome			
Anti-TB treatment	2 (20.00)	3 (100.00)	0.035
Outcome (death)	7 (70.00)	1 (33.33)	0.510
Significant time interval during TB development			
Duration of stay (Days)	22.00 (8.00–36.00)	71.00 (27.00–115.00)	0.028
Interval between symptom onset and hospital admission (Days)	35.00 (10.00–60.00)	10.00 (1.00–19.00)	0.469
Interval between hospital admission and TB diagnosis (Days)	28.00 (21.00–35.00)	31.50 (6.00–57.00)	0.007
Culture result turnaround time (Days)	22.50 (17.00–31.00)	26.00 (24.00–31.00)	0.371

Data are shown as *n* (%) or median (range).
Abbreviations: AFB, acid-fast-bacilli; BCG, Bacillus Calmette-Guérin; MTB, *Mycobacterium tuberculosis*; NK, natural killer; TB, tuberculosis; WBC, white blood cell.

diagnostic limitations, more sensitive methods and alternative samples may be needed to detect MTB complex bacteremia in children. Urine samples may be an alternative; a recent study found that urine Xpert MTB/RIF and urine lipoarabinomannan (LAM) identified 32 (78%) and 27 (66%), respectively, of the 41 MTB culture-positive blood samples in a population of HIV-infected inpatient adults in South Africa.[27,28] As BCG bacteremia is predominant in the lower age group, new diagnostic tools using multiple samples from children need to be explored.

Third, our data also showed important differences between MTB bacteremia patients and BCG bacteremia patients. We found that patients with BCG bacteremia were more likely to be younger, to suffer from primary immunodeficiency diseases, and have a lower level of CD4, IgA, and IgE. It is well known that the BCG vaccine is particularly efficacious against TB meningitis and miliary TB in children.[29] Before the 1920s when the BCG vaccine was first tested in humans, case reports of BCG bacteremia included children.[30] While the BCG vaccine likely plays a substantial role in preventing BCG bacteremia in children, there may be circumstances where the BCG vaccine may not be given or efficacious. The efficacy of the BCG vaccine is reduced in HIV-infected infants because the vaccine requires a T-cell response.[31] BCG bacteremia is a well known but uncommon complication of vaccination in immunocompromised persons.[32,33] However, most reported cases of BCG bacteremia were diagnosed months to years following vaccination and had high case fatality rates (60%–70%).[33] Based on our research results, if the following conditions are met there should be a high degree of clinical suspicion of BCG bacteremia to ensure an early diagnosis: (1) the patient's age is less than 3 years; (2) there are underlying sources of immunosuppression; (3) there was an adverse reaction after BCG vaccination, such as scar ulceration, lymphadenectasis, and fever.

Our study had several limitations. First, the number of patients with MTB complex bacteremia identified was still small although it had the largest sample size so far. This limited our ability to identify minor risk factors. Nonetheless, our study was conducted over 2 years and likely represents one of the largest studies comprehensively and multidimensionally exploring the characteristics of MTB complex bacteremia in a sample of HIV-negative children. Second, we did not develop an effective method for the rapid diagnosis of MTB complex bacteremia, and this will become the key emphasis in our future research. Although the systematic collection of samples for routine diagnosis of pulmonary tuberculosis was a component of the routine clinical care of study participants, it was not part of the study design, preventing assessment of sputum mycobacteriology as a predictor of disseminated tuberculosis. Furthermore, our study was not designed to comprehensively evaluate host and organism factors associated with MTB complex bacteremia.

In conclusion, MTB complex bacteremia is rapidly fatal in a large proportion of immunodeficient children. Classic findings, such as the clinical signs and derangements of basic hematologic and biochemical tests, may not be specific enough for the diagnosis: miliary shadowing on chest radiographs may be absent and culture-confirmation of

MTB complex bacteremia may not be available timeously. A high level of clinical suspicion should be maintained, especially in patients with certain sources of immunosuppression. In addition, there are significant differences between BCG bacteremia and MTB bacteremia patients. Studies are needed to develop and assess rapid diagnostic tests, and to determine the value of empirical therapy in MTB complex bacteremia.

CONFLICT OF INTEREST

Shunying Zhao, Gang Liu, and Huyong Zheng are members of the *Pediatric Investigation* editorial board.

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SUPPORTING INFORMATION

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How to cite this article: Chu P, Shi J, Dong F, Yang H, Zhao S, Liu G, et al. Bacteremia tuberculosis among HIV-negative children in China. *Pediatr Investig*. 2022;6:197–206. https://doi.org/10.1002/ped4.12342