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Point-of-Care Ultrasound for Extrapulmonary Tuberculosis in India: A Prospective Cohort Study in HIV-Positive and HIV-Negative Presumptive Tuberculosis Patients

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Abstract. Diagnosing extrapulmonary tuberculosis (EPTB) is challenging. Point-of-care ultrasound (POCUS) for human immunodeficiency virus (HIV)-associated EPTB is applied in sub-Saharan Africa. This study aimed at evaluating the applicability of POCUS for diagnosing EPTB in HIV-positive and HIV-negative presumptive tuberculosis (TB) patients in India, a country of moderate relative TB and HIV burden. Presumptive TB patients at Kasturba Hospital, Manipal, India, prospectively underwent POCUS evaluating for pericardial, pleural and ascitic effusion, abdominal lymphadenopathy, and hepatic and splenic microabscesses. Findings were correlated with TB category (confirmed TB, clinical TB, unlikely TB), HIV status, and discharge diagnoses. A total of 425 patients underwent POCUS; 81 (20%) were HIV-positive. POCUS findings were more common in HIV/TB coinfected patients than in HIV-positive patients with unlikely TB (24/40 (60%) versus 9/41 (22%), $P < 0.001$). Abdominal lymphadenopathy and splenic microabscesses were strongly associated with TB in HIV-positive patients ($P = 0.002$ and $P = 0.001$). POCUS findings did not correlate with TB in HIV-negative patients; a third of HIV-negative patients with unlikely TB and POCUS findings had cancer, another third other infectious diseases. Sonographic findings were common in HIV-positive and HIV-negative presumptive TB patients. POCUS was a useful bedside test for the detection of HIV-associated EPTB. In HIV-negative patients, POCUS detected features associated with EPTB but also of malignancy and other infectious diseases.

INTRODUCTION

Tuberculosis (TB) continues to substantially contribute to global morbidity and mortality. Case detection rates for extrapulmonary TB (EPTB) remain low because of unavailability of accurate point-of-care diagnostic tests.[1] EPTB affects many anatomical sites, and diagnosis often requires cross-sectional imaging. Human immunodeficiency virus (HIV)-positive patients and children are at increased risk for EPTB; however, HIV-negative patients also suffer from EPTB.[2–4]

In sub-Saharan countries, with pronounced relative HIV and TB burden, point-of-care ultrasound (POCUS; i.e., the Focused Assessment with Sonography for HIV-associated TB [FASH] protocol) is increasingly applied to support a timely diagnosis of EPTB.[5–8]

POCUS for EPTB has not been evaluated in populations outside the high HIV/TB prevalent setting of sub-Saharan Africa or in HIV-negative patients. In countries with lower TB prevalence, sonographic findings may relate to diseases other than TB; therefore, POCUS for EPTB cannot simply be transferred from one epidemiologic setting to another. India carries over a quarter of the global TB burden and accounts for most unreported or undiagnosed cases worldwide.[1] Relative prevalence of TB and HIV, however, is considerably lower than in most sub-Saharan countries. The aim of this study was to prospectively investigate the performance of POCUS for EPTB in Indian HIV-positive and HIV-positive patients with suspected TB.

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MATERIALS AND METHODS

This prospective controlled cohort study was conducted at the Department of Medicine and the Department of Pulmonary Medicine of Kasturba Medical College (KMC) in Manipal, India. The study was approved by the KMC and Kasturba Hospital Institutional Ethics Committee (registry number 563/2015). Written informed consent in the patient's language was obtained before any study procedure. Parents' or guardians' consent was obtained for minors and patients unable to give consent because of clinical circumstances.

Study population and TB work-up. Presumptive TB patients ≥ 16 years presenting at the Department of Medicine or the Department of Pulmonary Medicine were enrolled if a diagnostic TB workup was initiated by the hospital; therapeutic and diagnostic management was fully the responsibility of the attending hospital doctor. Routine TB workup commonly comprised one to three sputum examinations (fluorescence microscopy [culture and molecular testing were only performed in a minority of patients with special indications]), blood tests (basic hematology and clinical chemistry, HIV-testing, and CD4 cell count if applicable) and chest X-ray (CXR). Additional imaging studies, mycobacteriological or histological investigations on specimen other than sputum were performed in patients with suspected EPTB. CXRs were interpreted by a senior radiologist blinded to clinical and ultrasound data who categorized CXRs "suggestive of TB" or "not suggestive of TB."

For study purposes, patients were categorized into: "confirmed TB" (i.e., positive fluorescent microscopy, polymerase chain reaction, or TB culture), "clinical TB" (no microbiological confirmation, but clinical TB diagnosis and TB treatment initiated) and "unlikely TB" (no TB diagnosis and no TB

treatment; discharge diagnosis other than TB or no discharge diagnosis).

Evidence was considered sufficient for a "clinical TB" diagnosis if two of the criteria presented in Box 1 (TB diagnostic score) were met; TB diagnostic score collated for this study based on literature.[9–18]

If less than two criteria were met, TB was considered unlikely and the patient categorized as "unlikely TB." If a diagnosis other than TB was microbiologically or histologically confirmed despite the presence of two or more of the previously mentioned criteria and in the absence of microbiological TB confirmation, patients were also categorized "unlikely TB." Cases in which the hospital discharge diagnosis differed from the diagnosis given by the previously mentioned study score were excluded from analyses to improve case definition quality. Discharge diagnoses and affected TB sites were retrieved from medical records and documented as "PTB" (exclusively PTB or PTB with EPTB) or "EPTB" (exclusively EPTB). Patients were further categorized as "HIV-positive," "HIV-negative" (as per current HIV testing or HIV history) or "HIV status unknown."

For patients with a positive FASH examination, but with either clinical or unlikely diagnosis of TB, follow-up data were extracted from medical records or by contacting the patient via phone 6 weeks after enrolment; follow-up data included maintenance of discharge diagnosis, clinical outcome, and adherence to TB treatment.

Ultrasound methodology. All patients underwent a clinician-performed bedside ultrasound examination (without prior fasting) performed by a single operator who was trained in the study's ultrasound protocol but without formal ultrasound training. Two ultrasound devices were used: 1) Philips VISIQ™ with a C5-2 curved-array transducer (1–6 MHz) and 2) SonoSite® MicroMaxx with a curved-array (C60e, 5–2 MHz) and linear (L38e, 10–5 MHz) transducer. The first 171 patients were scanned with the Philip's VISIQ and the following 254 patients with the SonoSite's MicroMaxx device. A change in device was undertaken as the performance of the Philip's VISIQ for the applied protocol appeared limited.

The applied FASH protocol in summary assesses for pericardial, pleural and ascitic effusion, focal liver or splenic microabscesses, and abdominal lymphadenopathy.[5] A FASH examination was considered positive if at least one of the following findings was visualized: pericardial or pleural effusion, focal liver or splenic lesions, or abdominal lymphadenopathy. Ultrasound views were digitally stored and documented on standardized case report forms and disclosed to the attending physician. Images of 10% randomly selected patients (computer based, www.random.org) were reviewed by a second reader experienced in ultrasound and categorized as "FASH-positive" or "FASH-negative."

Data collection and analyses. Demographic and clinical data were collected from patient interviews and clinical examination; laboratory data were retrieved from the digital hospital laboratory system. Statistical analyses were performed using IBM SPSS Statistics® (Version 21). Continuous data were summarized by mean and standard deviation if normally distributed, otherwise by median and interquartile range. χ^2 test and median test were used two sided at $P = 0.05$. Cohen's kappa coefficient was calculated for the agreement of "FASH-positive" classification by the operator and second reader.

RESULTS

Five hundred and three patients were screened and 475 patients were enrolled between January 29 and July 2, 2016. Fifty patients were excluded from analyses because of insufficient data to allow categorization into TB categories. The final study cohort thus comprised 425 patients who were suspected of having TB at presentation.

Demographic, TB, and HIV data. Demographic, clinical TB, and HIV data are presented in Table 1.

Overall ultrasound data. Detailed ultrasound data are presented in Table 2. HIV-negative patients were as likely as HIV-positive patients to have FASH findings. Patients diagnosed with TB (either confirmed or clinical) were more likely to have a positive FASH examination than patients with "unlikely TB." In HIV-negative patients, a positive FASH examination was not associated with a diagnosis of TB; but in HIV-positive patients, a positive FASH examination was highly associated with TB diagnosis. HIV/TB coinfected patients had a significantly higher rate of positive FASH examinations than HIV-negative TB patients.

Ultrasound in HIV-positive patients. Whereas overall, positive FASH examinations correlated highly with TB

Box 1

Tuberculosis diagnostic score criteria (based on and adapted from references 9 to 18); patients with two or more criteria were categorized as "clinical tuberculosis"

Characteristic TB symptoms	PTB: cough ≥ 2 weeks or hemoptysis Lymph node TB: lymph node swelling ≥ 2 weeks Abdominal TB: abdominal distension, tenderness or diarrhea ≥ 2 weeks in the absence of chronic liver disease Central nervous system TB: altered mental status, focal neurological deficit or seizures Constitutional symptoms: fever in addition to at least one more constitutional symptom (night sweats, fatigue, weight loss) ≥ 2 weeks
Histopathology characteristic for TB	Granuloma, caseous necrosis or if the suspicion of TB was raised in the pathology report
Imaging characteristic for TB	CXR "suggestive of TB" Cross-sectional imaging reported as suggestive of TB
ADA level in fluid above cutoff	> 36 IU/l in pleural fluid > 39 IU/l in ascitic fluid > 10 IU/l in cerebrospinal fluid
Clinical response to anti-tuberculous treatment	Reduction of symptoms or radiological signs under anti-tuberculous treatment or Clinical deterioration in the absence of anti-tuberculous treatment

ADA = aminodeaminase; CXR = chest X-ray; PTB = pulmonary tuberculosis; TB = tuberculosis.

TABLE 1
Demographics and clinical data by tuberculosis category

	All	Confirmed or clinical TB	Confirmed TB		Clinical TB		Unlikely TB	PTB vs. unlikely TB
			PTB	EPTB	PTB	EPTB		
n (%)	425 (100)	285 (67)	163 (57)*	33 (12)	23 (8)†	66 (23)	140 (33)	
Demographics and TB History								
Male, *n* (%)	328 (77)	221 (78)	131 (80)	25 (76)	14 (61)	51 (77)	107 (76)	0.797
Age, median (IQR)	43 (31.5;55)	40 (30;51)	45 (32;55)	37 (30;43.5)	30 (26;50)	38 (26.8;49)	49 (39;60)	**0.000**
Previous TB, n (%)	81 (19)	55 (19)	40 (24)	5 (15)	5 (22)	5 (8)	36 (19)	0.858
BCG vaccinated, n (% of known)	335 (93)	231 (93)	132 (93)	26 (90)	19 (95)	54 (95)	104 (93)	0.921
HIV data								
HIV-positive, *n* (% of known)‡	81 (20)	40 (15)	10 (6)	14 (42)	4 (17)	12 (20)	41 (32)	**0.000**
CD4 cells/μL, median (IQR); *n* cases tested	112 (50;253); 73	112 (57;256.75); 36	113.5 (74.5;178.5); 10	100 (49.75;268.5); 14	(37, 51, 107)§	142 (94.5;438); 9	108 (36.5;238.5); 37	0.906‖
ART, *n* (%)¶	29 (66)	14 (64)	3 (50)	5 (71)	1 (50)	5 (71)	15 (68)	0.750
Symptoms								
Cough, *n* (%)	340 (80)	232 (81)	155 (95)	15 (46)	22 (96)	40; (61)	108 (77)	0.302
Fatigue, *n* (%)	328 (77)	220 (77)	125 (77)	25 (76)	18 (78)	52 (79)	108 (77)	0.991
Fever, *n* (%)	286 (67)	197 (69)	108 (66)	22 (67)	17 (74)	50 (76)	89 (64)	0.252
Weight loss, *n* (%)	283 (67)	200 (70)	124 (76)	19 (58)	17 (74)	40 (61)	83 (59)	**0.025**
Abdominal symptoms, *n* (%)	151 (36)	103 (36)	50 (31)	14 (42)	11 (48)	28 (42)	48 (34)	0.707
Thoracic pain/dyspnea, *n* (%)	86 (20)	51 (18)	29 (18)	4 (12)	0	18 (27)	35 (25)	0.087
Lymph node swelling, *n* (%)	29 (7)	25 (9)	2 (1)	10 (30)	1 (4)	12 (18)	4 (3)	**0.023**
CXR								
CXR suggestive of TB, *n* (%)	198 (47)	177 (63)	144 (88)	7 (23)	13 (57)	13 (20)	21 (15)	**0.000#**

ART = anti-retroviral therapy; CXR = chest X-ray; *n* = number of cases; EPTB = extrapulmonary tuberculosis without PTB; HIV = human immunodeficiency virus; IQR = interquartile range; PTB = pulmonary tuberculosis incl. cases with concurrent EPTB; TB = tuberculosis. Statistically significant results are marked in bold ($P < 0.05$).

* Eighteen (11%) of 163 patients with confirmed PTB had a concurrent EPTB diagnosis, of these 6/18 (33%) were HIV positive.

† Twelve (52%) of 23 patients with clinically diagnosed PTB had a concurrent EPTB diagnosis and 3/12 (25%) were HIV positive.

‡ HIV status could not be ascertained in 24 (6%) patients.

§ $N = 3$, absolute CD4 cell counts.

‖ P value for PTB vs. EPTB: $P = 1.0$.

¶ Median time on ART (IQR 0.3;7) years.

P value for PTB vs. unlikely TB $P < 0.001$.

TABLE 2
Sonographic findings in all patients and patients stratified by tuberculosis category and HIV status

	All								HIV-positive				HIV-negative				All	TB
		TB																
			Confirmed TB		Clinical TB													
	All cases	All	PTB	EPTB	PTB	EPTB	Unlikely TB	PTB vs. unlikely TB	All	TB	Unlikely TB	PTB vs. unlikely TB	All	TB	Unlikely TB	PTB vs. unlikely TB	*P* HIV-positive vs. HIV-negative	*P* HIV-positive vs. HIV-negative
n (%)	425	285 (67)	163 (57)	33 (12)	23 (8)	66 (23)	140 (33)		81	40 (49)	41 (51)		320	233 (73)	87 (27)			
Pericardial effusion, *n* (%)	14* (3)	10 (4)	5 (3)	1 (3)	0	4 (6)	4 (3)	1.000	5 (6)	5 (13)	0	0.055	9 (3)	5 (2)	4 (5)	0.256	0.185	**0.011**
Pleural effusion, *n* (%)	104†,‡ (24)	78 (27)§	26 (16)	7 (21)	6 (26)	39 (59)	26 (19)	**0.047**	7 (9)	5 (13)	2 (5)	0.265	91 (28)	70 (30)	21 (24)	0.297	**<0.001**	**0.022**
Focal liver lesions, *n* (%)	5 (2)	4 (1)	1 (1)	0	1 (4)	2 (3)	1 (1)	0.669	2 (3)	1 (3)	1 (2)	1.000	3 (1)	3 (1)	0	0.564	0.275	0.481
Focal spleen lesions, *n* (%)	16 (4)	14 (5)	2 (1)	7 (21)	3 (13)	2 (3)	2 (1)	0.078	13 (16)	12 (30)	1 (2)	**0.001**	3 (1)	2 (1)	1 (1)	1.000	**<0.001**	**<0.001**
Abdominal lymphadenopathy, *n* (%)	49‖ (12)	39 (14)	14 (9)	12 (36)	5 (24)	8 (12)	10 (7)	**0.047**	25 (31)	19 (48)	6 (15)	**0.002**	24 (8)	20 (9)	4 (5)	0.556	**<0.001**	**<0.001**
Ascites, *n* (%)	27¶ (6)	21 (7)	11 (7)	1 (3)	3 (13)	6 (9)	6 (4)	0.221	3 (4)	2 (5)	1 (2)	0.616	23 (7)	18 (8)	5 (6)	0.542	0.255	0.748
FASH positive, *n* (%)	156 (37)	118 (41)	37 (23)	21 (64)	12 (52)	48 (73)	38 (27)	**0.004**	33 (41)	24 (60)	9 (22)	**<0.001**	117 (37)	91 (39)	26 (30)	0.130	0.488	**0.013**

EPTB = extrapulmonary tuberculosis without PTB; FASH = focused assessment with sonography for HIV-associated tuberculosis; FASH positive = one or more FASH findings visualized; HIV = human immunodeficiency virus; *n* = number of cases with respective characteristic; PTB = pulmonary tuberculosis incl. cases with concurrent EPTB; TB = tuberculosis. Statistically significant results are marked in bold ($P < 0.05$).

* Cardiomegaly reported in chest X-ray in $N = 0$.

† Pleural effusion reported in chest X-ray in 85 (82%).

‡ TB diagnosis was not more common in right or left sided of pleural effusions (43/56 (77%) right sided, 27/37 (73%) left sided, and 8/11 (73%) bilaterally located pleural effusions were diagnosed with TB, $P = 0.902$).

§ Forty-three (61%) of 70 unilateral pleural effusions in patients with TB were located on the right side, $P = 0.677$.

‖ In 32/49 (65%), lymph nodes were visualized in the periportal region, in 23/49 (47%) in the mesenteric/paraaortic region, in 22/49 (45%) in the parapancreatic region, and in 3/49 (6%) in the perisplenic region.

¶ Ascites was visualized in the rectovesical/Douglas pouch in 21/27 (78%), the hepatorenal pouch in 15 (56%), and the perisplenic region in 15 (56%) patients.

diagnosis in HIV-positive patients, not all individual FASH findings correlated with TB diagnosis (Table 2). Abdominal lymphadenopathy and splenic microabscesses were the most common FASH findings in HIV/TB coinfected patients and were the only FASH findings statistically associated with TB diagnosis. Most FASH findings in HIV-positive patients with "unlikely TB" could not be related to specific differential diagnoses (Table 3).

Of the 13 HIV-positive patients clinically diagnosed with TB and whose CXR were not suggestive of TB, five (39%) had a positive FASH examination (abdominal lymphadenopathy $N = 4$, splenic microabscesses $N = 3$ patients).

In HIV-positive patients diagnosed with PTB or EPTB (confirmed or clinical diagnosis), the FASH examination was positive in 8/14 (57%) and 16/26 (62%), respectively. The HIV-positive patients with a negative FASH examination who were diagnosed with EPTB ($N = 13$) had the following EPTB manifestations: (extra-abdominal) tuberculous lymphadenitis ($N = 8$), cerebral TB ($N = 2$), disseminated TB ($N = 2$, locations not further specified), and musculoskeletal TB ($N = 1$).

Stratification of HIV/TB coinfected patients by CD4 cell count above or below 100 cells/μL did not show differing rates of single FASH findings or positive FASH examinations; median CD4 cell count was similar in patients with a positive or negative FASH examination ($P > 0.05$).

Ultrasound in HIV-negative patients. In HIV-negative patients, the rate of positive FASH examinations did not differ between patients diagnosed with TB or with "unlikely TB."

Neither individual FASH finding was associated with TB diagnosis in HIV-negative patients. In HIV-negative patients, pleural effusion was by far the most common FASH finding, followed by abdominal lymphadenopathy and ascites (Table 2). Splenic microabscesses, abdominal lymphadenopathy, and pericardial effusion were significantly less common in HIV-negative TB patients compared with HIV/TB coinfected patients (Table 2). Inversely, pleural effusions were significantly more common in HIV-negative TB patients compared with HIV/TB coinfected cases. HIV-negative patients with confirmed PTB had a positive FASH examination in 32/146 (22%) cases (pleural effusion in 23, abdominal lymphadenopathy in 9, and pericardial effusion in 3 cases).

Eight of 26 (31%) HIV-negative "unlikely TB" patients who had a positive FASH examination, were diagnosed with a malignancy, 8/26 (31%) with an infectious disease other than TB, 7/26 (27%) had a discharge diagnosis of a chronic noninfectious and nonmalignant disease, and 3/26 (12%) were discharged without any specific diagnosis (Table 3).

Additive ultrasound value in cases with confirmed PTB. Of 163 patients with confirmed PTB, 18 (11%) had concurrent PTB and EPTB diagnosed by hospital physicians. In 24/145 (17%) patients with exclusive PTB, FASH examination was positive suggesting concurrent PTB and EPTB; FASH thereby increased the number of patients with concurrent PTB and EPTB by 15% to a total of 26%.

Sonographic incidental findings. The following incidental sonographic findings not targeted for by the FASH protocol were detected during FASH examinations: fatty infiltration of the liver; hepatic metastases, abscesses, and cysts, portal vein thrombosis; cholelithiasis; splenomegaly; splenic infarctions; splenic calcified lesions; hydronephrosis; polycystic kidney disease; intestinal wall thickening; and aortic aneurysm.

Sonographic quality. Ultrasound images of all 12 FASH views were obtained for all 425 patients. Of these, 342 (7%) were categorized as "not evaluable" (most commonly views for lymphadenopathy, followed by pericardial effusion and splenic microabscesses). External validation of randomly selected still images showed a 96% agreement with "FASH-positive" or

TABLE 3

Final discharge diagnoses of patients with sonographic findings and unlikely tuberculosis stratified by HIV status (multiple sonographic findings per patient possible)

	Total	HIV-positive	HIV-negative	HIV status not determined
Pericardial effusion	4	–	3 malignancy* 1 *Klebsiella* pneumonia	–
Pleural effusion	26	1 *Acinetobacter* pneumonia 1 unspecified[a]	7 malignancy† 2 parapneumonic effusion 2 anemia[a] 1 acute respiratory tract infection 1 post-TB residuae 1 interstitial lung disease 1 chronic hypersensitivity pneumonitis 1 *Klebsiella* empyema 1 Kikuchi's disease 1 spinal canal stenosis[a] 3 unspecified[a]	1 parapneumonic effusion 1 post-TB residuae 1 allergic rhinitis[a]
Focal liver lesions	1	1 pyelonephritis[a]	–	–
Focal spleen lesions	2	1 unspecified[a]	1 brucellosis	–
Lymph nodes	10	1 acute respiratory tract infection[a] 1 acute gastroenteritis 4 unspecified[a]	2 brucellosis 1 Kikuchi's disease 1 lymphoma	–
Ascites	6	1 acute respiratory tract infection	1 malignancy‡ 1 ischemic heart disease[a] 1 Kikuchi's disease[a] 2 unspecified[a]	–

HIV = human immunodeficiency virus; SIADH = syndrome of inappropriate antidiuretic hormone secretion; TB = tuberculosis.

* Two cases of adenocarcinoma of the lung (histopathological confirmation), one case with radiological diagnosis of pulmonary neoplasia.

† Three cases of adenocarcinoma of the lung, one case with non-small cell lung cancer (histopathological confirmation), three cases with radiological diagnosis of pulmonary neoplasia (including one case with SIADH).

‡ Cells of adenocarcinoma detected in peritoneal fluid.

[a] final discharge diagnosis without relation to sonographic finding and no specific differential diagnosis for sonographic finding identified.

"FASH-negative" classification and a Cohen's kappa coefficient of 0.919.

DISCUSSION

This is the first study to evaluate POCUS for EPTB in presumptive TB patients in India. TB was a frequent diagnosis in this study setting with around two thirds of presumptive TB subjects being diagnosed with active TB. The HIV-infection rate was relevant with every sixth TB patient being HIV coinfected, in line with previous reports from the study region.[19] POCUS yielded sonographic findings in more than a third of both HIV-positive and HIV-negative presumptive TB subjects. However, whereas in HIV-positive patients, a positive FASH examination correlated with TB, in HIV-negative patients, a positive FASH examination did not correlate with TB.

The overall association of a positive FASH examination with TB diagnosis in HIV-positive patients was driven by the two most common ultrasound findings, abdominal lymphadenopathy, and splenic microabscesses, both of which were strongly associated with TB diagnosis. Abdominal lymphadenopathy and splenic microabscesses were also frequent sonographic findings reported from HIV/TB coinfected patients from other settings.[20–23]

For pericardial, pleural, and ascitic effusions, as well as focal liver lesions, no association with TB diagnosis was observed; however, it is important to note that numbers were low. In our study, sonographic findings were independent from CD4 T-cell counts. More data are needed to better understand the relation between the degree of immunosuppression and the risk for specific EPTB manifestations; other TB-related point-of-care tests have shown CD4 T-cell correlation.[24] In the subgroup of HIV-positive patients with clinical TB whose CXR examinations did not show changes suggestive of TB, POCUS provided sonographic evidence for TB (splenic microabscesses and abdominal lymphadenopathy) in more than a third of patients. This finding underlines the previously reported incremental diagnostic benefit of POCUS for this patient group.[20] Therefore, our data support the diagnostic value of POCUS for EPTB in HIV-positive presumptive TB patients in this Indian setting despite there being a lower HIV and TB prevalence than in sub-Saharan Africa.

This study provides the first prospective sonographic evaluation for EPTB in HIV-negative presumptive TB subjects; although sonographic findings were common, neither individual sonographic findings nor a positive FASH examination were associated with TB diagnosis. Pleural effusion was the most common finding in HIV-negative TB patients and far more common than in HIV-positive TB patients. A variety of diagnoses were found in patients with a positive FASH examination but "unlikely TB," underlining the nonspecific presentation of EPTB and its associated diagnostic challenge. Although the FASH examination had less of a predictive value for EPTB in HIV-negative than in HIV-positive patients, our data suggest that POCUS may still be supportive in establishing a diagnosis in these patients. In the study cohort, a malignant disease was diagnosed in around a third of HIV-negative patients with "unlikely TB" who had sonographic findings (i.e., pleural or pericardial effusion, lymphadenopathy). Brucellosis was a confirmed diagnosis in some patients presenting with abdominal lymphadenopathy or splenic microabscesses, previously described characteristic sonographic pattern of brucellosis[25] (Figure 1). Moreover, sonographic chance findings may reveal other relevant underlying conditions, as seen in this study.

Most previous studies evaluating the FASH protocol lacked a prospective approach or control groups[5,8,20,26]; one prospective controlled cohort study has recently been performed in pediatric patients from South Africa.[23] Data from the present and the previous studies suggest that the yield of scanning for focal liver lesions is marginal with respect to the detection of EPTB. Given that hepatic TB may not specifically present with microabscesses[27] and that sonographic liver assessment requires rather advanced ultrasound skills,[8] omitting liver assessment from a revised FASH protocol may render the protocol more concise for EPTB. The FASH protocol includes ascites as a characteristic feature of tuberculous peritonitis, but ascites without other features suggestive of abdominal TB was never considered sufficient evidence for EPTB; data from this study support this. Sonographic assessment for ascites, however, is easy to perform and assessment for ascites may be justified, because the awareness thereof may guide further diagnostic work-up. For an improved guidance on interpretation of pleural and pericardial effusion, more controlled studies from different epidemiologic settings are needed. The value of routine abdominal ultrasound for the detection of HIV-associated EPTB has been reported in a previous study from India[28]; however, whether and for which target population FASH can be a suitable protocol for integration into Indian POCUS curricula, as in sub-Saharan Africa,[6,8] will need further evaluation.

Little is known about EPTB pathophysiology and epidemiology, including concurrent PTB and EPTB. This prospective and controlled study showed that HIV infection increased the rate of concurrent EPTB in patients with confirmed PTB more than two-fold, thereby clearly supporting HIV as a risk factor for EPTB manifestations.[29] Moreover, every fifth HIV-negative patient with confirmed PTB had sonographic features

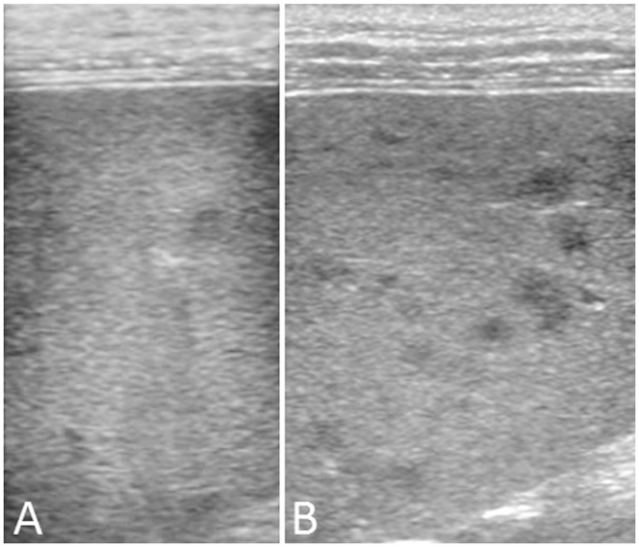

FIGURE 1. (**A**) Focal hypoechoic spleen lesions in an human immunodeficiency virus (HIV)-negative patient with serologically confirmed Brucellosis and unlikely tuberculosis (TB). (**B**) Multiple hypoechoic splenic lesions in an HIV-positive patient with confirmed disseminated TB.

compatible with EPTB, mostly pleural effusions of varying size and abdominal lymphadenopathy. The rate of concurrent PTB and EPTB in HIV-negative patients may therefore be higher than previously assumed.

This study has some limitations. The lack of a high-frequency linear probe while the first ultrasound device was used may have led to an underdetection of splenic microabscesses and mesenteric lymphadenopathy; spleen parenchyma irregularities are more accurately detected with higher than with lower frequency probes.[30] Also, the true rate of abdominal lymphadenopathy may have been underestimated because of unfavorable scanning conditions in some patients. Sonographic findings were not microbiologically or histologically confirmed as EPTB, which would have required clinically and ethically unjustified invasive diagnostic procedures. Whether some of the (smaller) pleural effusions were parainfectious rather than true tuberculous pleuritis therefore remains unknown. For some patients with sonographic findings, no diagnosis was established precluding interpretation of these findings. Sonographic follow-up was not performed in this study; the evolution of sonographic findings with or without TB treatment may have added further evidence on the etiology of sonographic findings.

Notably, the present study is the first prospective and controlled evaluation of POCUS in HIV-positive and HIV-negative adults. Furthermore, TB case definition quality was ensured by applying a stringent algorithm and sonographic and CXR data quality was ensured by external validation.

CONCLUSIONS

The performance of POCUS for detecting EPTB in patients suspected of having TB depended on the patient's HIV status. A positive ultrasound examination, the detection of abdominal lymphadenopathy and splenic microabscesses in particular, correlated strongly with TB diagnosis in HIV-positive patients. Therefore, this study suggests that POCUS can support the diagnosis of HIV-associated EPTB in epidemiologic settings with moderate TB and HIV prevalence. In HIV-negative patients, neither a positive ultrasound examination nor individual sonographic findings correlated with TB diagnosis; however, sonographic findings were common in patients with TB, malignancy, or other infectious diseases. Interpretation of ultrasound findings must therefore take into account the patient's HIV status and entail awareness for possible differential diagnoses.

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