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Diagnostic accuracy of sonographic signs of extrapulmonary tuberculosis and treatment response monitoring in HIV-positive and -negative populations

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ABSTRACT

Background: Diagnosis and monitoring of extrapulmonary tuberculosis (EPTB) remains challenging. Ultrasound such as the extended focused assessment for HIV-associated tuberculosis (eFASH) protocol might improve diagnosis and monitoring of treatment response. This study determined the diagnostic accuracy of eFASH for EPTB and its value in monitoring EPTB treatment response compared with clinical signs and symptoms.

Methods: We performed a post-hoc analysis of a trial assessing the impact of eFASH on management of adults with suspected EPTB. Participants with baseline and follow-up ultrasound examinations were included. We assessed the diagnostic accuracy of eFASH and compared the 2-month evolution of eFASH findings and clinical signs and symptoms among participants with definite EPTB, stratified by favorable treatment outcomes at 6 months.

Results: In 296 included participants (95 with definite EPTB, 201 with no definite EPTB), the most common eFASH signs were pleural effusion (47%) and pulmonary B-lines with subpleural granular artefacts (34%). Pleural effusion was the only sign that persisted beyond 6 months. eFASH had a sensitivity of 93.7% (95% CI, 86.8-97.6) and a specificity of 37.8% (95% CI, 31.1-44.9) for definite EPTB. At 2 months, favorable outcomes were similar between participants with full and partial resolution of eFASH signs (83% versus 81%). In contrast, a higher proportion of favorable outcomes was seen in participants with full resolution of clinical signs and symptoms (90% versus 60%).

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Conclusion: eFASH shows high sensitivity but low specificity for diagnosis of definite EPTB. Beyond diagnosis, ultrasound may complement clinical assessment for monitoring treatment response in EPTB.

Key words: eFASH, FASH, ultrasound, extra-pulmonary tuberculosis, treatment monitoring

Background

Tuberculosis (TB) remains the leading cause of death from a single infectious agent, causing almost double the number of deaths from HIV/AIDS. An estimated 10.8 million people fell ill with TB in the year 2023, with 1.2 million succumbing to the illness (1). Despite being primarily a pulmonary disease (pulmonary tuberculosis (PTB)), *Mycobacterium tuberculosis* can affect other organs in the form of extrapulmonary tuberculosis (EPTB) (2). EPTB contributes to 15-25% of TB cases worldwide and as many as 50% of TB cases in the immunocompromised population (3). In 2023, it accounted for 16% of newly diagnosed TB cases[1]. EPTB is microbiologically confirmed in only a quarter of cases (4).

The diagnosis of EPTB remains challenging, with lower microbiological confirmation rates in extrapulmonary samples compared to sputum samples and the need for invasive sample collection procedures (4-6). A recent Cochrane review reported a wide variation in the pooled sensitivity (63%-68%) and specificity (68%-73%) of ultrasound in the diagnosis of EPTB, depending on whether bacterial confirmation or clinical diagnosis was used as the reference standard (7). The presence of multiple positive ultrasonographic signs of EPTB increased the specificity of ultrasound in one study (8).

Monitoring EPTB treatment response also remains difficult. Microbiologic methods such as Xpert MTB/RIF® can remain positive for months to years despite successful treatment (9). Therefore, the clinical assessment of signs and symptoms is mostly utilized to monitor treatment response (10, 11). Information on the usefulness of ultrasound for monitoring TB treatment outcomes in patients with EPTB is limited. A single-center case series on the changes in an ultrasound point-of-care protocol (focused assessment for HIV-associated TB (FASH)) among 21 HIV and TB-coinfected patients at 1, 3, 6 and 12 months after the initiation of anti-tuberculosis treatment showed that most of the ultrasound findings resolved within 3 months; splenic abscesses and abdominal lymph nodes were the most common persisting findings (12). Another study showed that baseline and 3-month ultrasound findings of mediastinal lymphadenopathy are useful for diagnosing TB and monitoring treatment response in children (13).

In a previous randomized clinical trial, we developed an extended FASH (eFASH) protocol that incorporated ultrasonographic assessment of the chest and inferior vena cava as indicators of heart failure, as well as the evaluation of the cervical and axillary lymph nodes, into the existing FASH protocol (14). While eFASH, in addition to microbiological tests, did not improve the overall correct management of patients suspected of having EPTB, it improved the proportion of microbiologically confirmed cases of EPTB from 24% to 35% through safe invasive sample collection procedures (5). However, it remained unclear whether the additional eFASH signs enhanced the diagnostic performance of the original FASH protocol or whether changes in these findings over time could be used to monitor treatment response. Here, we report the diagnostic accuracy of eFASH signs, their evolution during treatment, and their role in monitoring treatment response in patients with microbiologically confirmed EPTB, compared with clinical findings.

Methods

Study design and population

We conducted a post-hoc analysis of data from a randomized two-center (one rural and one urban regional referral hospital in Tanzania) trial conducted

from September 2018 to October 2020, that assessed the use of ultrasound in the correct management of 701 participants (aged ≥15 years) suspected of having EPTB (5, 14). An eFASH examination was performed at enrolment and at 2 and 6 months of follow-up in the intervention arm, in addition to clinical evaluation.

eFASH performance and interpretation

All ultrasound examinations were performed by three point-of-care sonographers certified by the European Federation of Societies for Ultrasound in Medicine and Biology using a Sonobook 9 ultrasound system (Chison, Jiangsu, China), with a 3.0-MHz convex probe and a 7.5-MHz linear probe. The sonographers received two hours of training in the eFASH protocol prior to study initiation. An independent board-certified sonographer remotely reviewed baseline eFASH examinations (5). Image acquisition and interpretation followed the standard FASH protocol (15), with selected extended examinations as previously described (14) and further detailed in Supplementary Table S1 and Supplementary Figure S1.

Reference standard and case definitions

Microbiological confirmation of TB was performed using Xpert MTB/RIF Ultra® (Cepheid, Sunnyvale, CA) in all samples except sputum, which was tested by conventional Xpert MTB/RIF®, the standard test at the time of the study. Other tests included culture for all samples except urine; adenosine deaminase (ADA) testing in ascitic, pericardial and pleural fluid; and fine needle aspiration cytology for lymph nodes (5, 14).

Diagnostic accuracy analysis

For the diagnostic accuracy analysis, we included participants with suspected EPTB who had a baseline eFASH examination and sufficient reference standard data to allow definitive classification. This analysis comprised 296 participants including 95 with definite EPTB and 201 without definite EPTB.

Longitudinal ultrasound analysis

To evaluate the evolution of eFASH signs during anti-TB treatment, we included 21 participants with definite EPTB who had at least one positive eFASH sign at baseline and available follow-up ultrasound examinations at 2 and 6 months. This group represented a subset of the 28 participants with baseline, 2-month, and 6-month eFASH examination shown in Figure 1. A total of 7/28 participants had no eFASH signs at baseline. The smaller sample size reflects the requirement for both baseline ultrasound abnormalities and complete follow-up imaging.

Clinical follow-up analysis

For comparison of eFASH signs with clinical evolution, we analyzed 43 participants with definite EPTB who had documented baseline and 2-month clinical assessments. Differences in sample size between analyses were due to variation in availability of follow-up data and the presence of baseline eFASH abnormalities.

Definitions of eFASH positivity and outcome measures

Definite EPTB was defined as the presence of positive microbiology, histology, cytology, culture, or ADA (≥40 U/mL in pleural fluid, or ≥35 U/mL in ascites and pericardial fluid) in any extrapulmonary sample (Supplementary Table S2). Microbiologically unconfirmed (i.e., probable) EPTB, and no EPTB diagnoses were determined by the study clinician based on the microbiological, clinical, radiological and sonographic findings at baseline and follow-up with respect to tuberculosis treatment. We categorized participants with no TB, probable or definite PTB only, and those with probable EPTB as having no definite EPTB. A positive eFASH ultrasound examination was defined as the presence of at least one of the following: hypoechogenic lesions in the liver or spleen; pericardial effusion; pleural effusion in the absence of clinical or sonographic signs of heart failure (i.e., the presence of a non-dilated collapsing inferior vena cava); pulmonary B-lines together with subpleural granular artefacts; enlarged axillary, abdominal, nuchal or cervical lymph

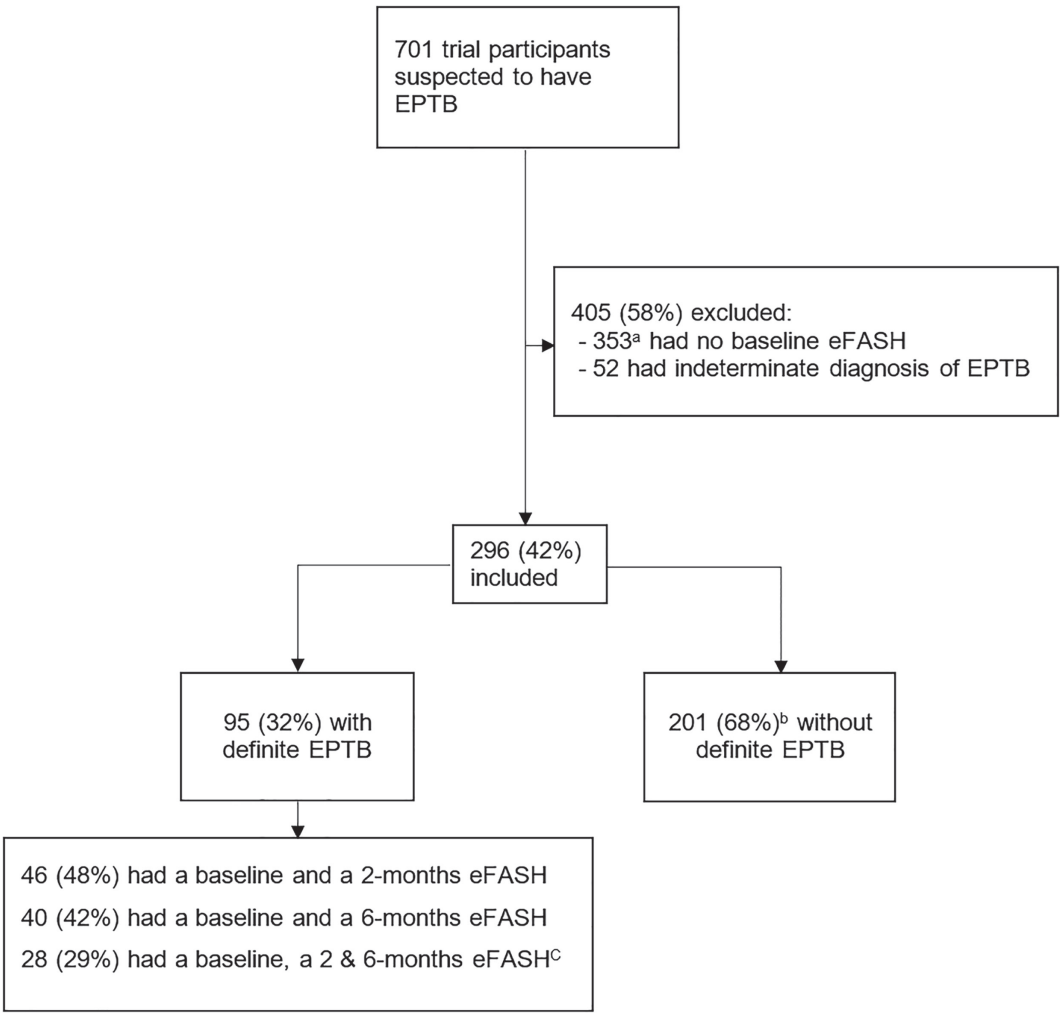

Figure 1. Participants' flow chart. [a]351 of the 353 participants were enrolled in the control arm without ultrasound. Two participants were from the intervention arm. [b]111 had probable EPTB, 90 had no EPTB. [c]Including participants who had a baseline and a 2-month or 6-month eFASH only. *Abbreviations:* EPTB, extrapulmonary tuberculosis; eFASH, extended focused assessment with sonography for HIV-associated tuberculosis.

nodes greater than 1.5 cm with no other explanation; an ileum wall >4 mm with loss of wall architecture and at least one other eFASH sign; abdominal ascites and at least one other eFASH sign (14).

Assessment of resolution and outcomes

Resolution of eFASH findings was categorized as completely resolved, partially resolved, or not changed or worsening based on comparison with the previous – baseline or 2 month – examination. Complete resolution meant disappearance of effusions or focal lesions, normalization of bowel wall thickness, and absence of ascites, lymphadenopathy, or pulmonary B-lines with subpleural granular artefacts. Partial resolution was defined as a ≥50% reduction in pleural effusion volume, pericardial effusion diameter, lymph node or lesion size, terminal ileum wall thickening, or in the number of pulmonary B-lines with subpleural granular artefacts. No resolution was defined as persistence, <50% reduction, or worsening of findings. Resolution of clinical signs and symptoms was categorized as fully resolved, partially resolved, or unchanged/worsened based on clinicians' assessment after physical examination and history taking. A favorable outcome

was assessed at 6 months and defined as being alive and having completed or continuing TB treatment with resolved or improved symptoms. Participants who died, were lost to follow-up, or had worsening or non-improved symptoms were classified as having an unfavorable outcome. A window of +/- 3 weeks for the 2-month follow-up visit and +/- 4 weeks for the 6-month follow-up visit was allowed.

Statistical analysis

Baseline demographics, clinical characteristics, and eFASH signs were compared between participants with definite and non-definite EPTB. Frequencies and percentages were used to express categorical variables, and medians with interquartile ranges (IQRs) for continuous variables. The sensitivity, specificity, negative and positive predictive value of eFASH signs were compared against the reference standard of definite tuberculosis. Inter-rater reliability of baseline eFASH findings was assessed by comparing the first sonographer's evaluations with those of an independent reviewer using overall agreement and Cohen's κ (16); assessments classified as indeterminate by the reviewer were treated as missing and excluded (Supplementary Table S3). The evolution of eFASH signs was described in participants with baseline, 2-month and 6-month eFASH examinations. Analyses were done in Microsoft Excel and Stata software version 16 for Windows (StataCorp., College Station, TX).

Results

Out of 701 participants enrolled in the eFASH trial, 405 were excluded (353 had no baseline ultrasound, 52 had an indeterminate diagnosis), leading to 296 participants, who were included in the final analysis. Of these, 95 participants had definite EPTB and 201 no definite EPTB (111 with probable EPTB and 90 with no EPTB) (Figure 1). Among participants with definite EPTB (n=95), 28 had ultrasound examinations at baseline, 2 months and 6 months; of these, 21 had at least one positive eFASH sign at baseline. The median age was 39 years (IQR 28-50). The majority of participants were male (n=174; 59%), HIV-negative (n=186, 63%), and had World Health Organization (WHO) screening symptoms (i.e., any cough (n=234, 79%), fever (n=237, 80%), night sweats (n=147, 50%), or weight loss (n=275, 93%) (17). All baseline eFASH findings were more common in participants with definite EPTB except for a thickened ileum wall (1% vs 1%) (Table 1). Among all 296 participants, 214 (72%) had a positive eFASH as defined above. The distribution of eFASH signs at baseline is shown in Supplementary Table S4, with pleural effusion (n=138, 47%) and pulmonary B-lines with subpleural granular artefacts (n=101, 34%) being the most common eFASH signs. Definite EPTB was diagnosed mostly by positive microbiology from pleural fluid (n=32, 22%) and urine (n=34, 23%) (Supplementary Table S2). A majority of the participants with definite EPTB (n=64, 67%) had a favorable treatment outcome. eFASH interpretations showed strong inter-rater reliability, with a 94% overall agreement between the site investigator and reviewer and a Cohen's κ of 0.84.

Diagnostic accuracy of eFASH

Table 2 shows the diagnostic accuracy of eFASH overall and of individual eFASH signs for definite EPTB. A positive eFASH had a sensitivity of 93.7 (95% confidence interval (CI), 86.8-97.6), a specificity of 37.8 (95% CI, 31.1-44.9), a negative predictive value of 92.7 (84.8-97.3) and a positive predictive value of 41.6 (95% CI, 34.9-48.5) for the diagnosis of definite EPTB. Hypo-echogenic lesions in the liver, spleen, pericardial effusion, and abdominal lymphadenopathy were present in 3%, 5%, 10%, and 16% of all 296 participants, respectively (Supplementary Table S4), and had a good specificity for definite EPTB while having limited sensitivity. The presence of pulmonary B lines together with subpleural granular artefacts had a sensitivity of 35.8 (95% CI, 26.2-46.3) and a specificity of 66.7 (95% CI, 59.7-73.1). A higher number of positive eFASH signs increased the specificity of eFASH to 98.5% (95% CI, 95.7-99.7) if more than 3 signs were present, with a reduced sensitivity of 9.4% (95% CI, 4.4-17.2) (Supplementary Table S5).

Table 1. Baseline characteristics of participants with and without definite EPTB.

Baseline Characteristics	Total	Definite EPTB[a]	No Definite EPTB[b]
	N=296	N=95	N=201
Demographic			
Age, median (interquartile range (IQR)	39 (28-50)	32 (25-43)	41 (29-52)
Sex, male, n (%)	174 (59%)	55 (58%)	119 (59%)
BMI (kg/m^2), median (IQR)	19.6 (17.9-22.2)	19.03 (17.4-20.9)	20 (18.2-22.6)
Medical History, n (%)			
HIV-positive	110 (37%)	44 (46%)	66 (33%)
History of previous tuberculosis	32 (11%)	3 (3%)	29 (14%)
WHO four symptoms screening, n (%)			
Any Cough	234 (79%)	70 (74%)	164 (82%)
Fever	237 (80%)	76 (80%)	161 (80%)
Night Sweats	147 (50%)	52 (55%)	95 (47%)
Weight Loss	275 (93%)	91 (96%)	184 (92%)
Other Symptoms, n (%)			
Haemoptysis	12 (4%)	3 (3%)	9 (4%)
Dyspnea	167 (56%)	56 (59%)	111 (55%)
Chest pain	207 (70%)	64 (67%)	143 (71%)
Neurological symptoms	19 (6%)	6 (6%)	13 (6%)
Urological symptoms	24 (8%)	5 (5%)	19 (9%)
Clinical signs, n (%)			
Pulmonary Signs	231 (78%)	74 (78%)	157 (78%)
Cardiac Signs	245 (83%)	83 (87%)	162 (81%)
Neurological signs	7 (2%)	4 (4%)	3 (1%)
Lymphadenopathy	106 (36%)	50 (53%)	56 (28%)
Abdominal Signs	139 (47%)	51 (54%)	88 (44%)
eFASH Signs, n (%)			
Pleural effusion	138 (47%)	52 (55%)	86 (43%)
Pericardial effusion	31 (10%)	16 (17%)	15 (7%)
Splenic hypo-echogenic lesions	16 (5%)	11 (12%)	5 (2%)
Liver hypo-echogenic lesions	8 (3%)	7 (7%)	1 (0%)
Ascites	67 (23%)	26 (27%)	41 (20%)
Abdominal Lymphadenopathy: • Para aortal >1.5cm3 • Mesenteric >1.5cm3	40 (14%) 23 (8%)	24 (25%) 15 (16%)	16 (8%) 8 (4%)
Thickened ileum wall >4 mm	3 (1%)	1 (1%)	2 (1%)
Pulmonary B lines with subpleural granular artefacts	101 (34%)	34 (36%)	67 (33%)

[a]Thirty participants had definite PTB in addition to definite EPTB, and 5 had probable PTB, in addition to definite EPTB; [b]21 had probable EPTB in addition to definite PTB, 8 had only definite PTB, 7 had probable EPTB and probable PTB, 3 had only probable PTB, 83 had only probable EPTB, and 79 had neither PTB nor EPTB. *Abbreviations:* EPTB, Extrapulmonary Tuberculosis; eFASH, extended focused assessment with sonography for HIV-associated tuberculosis.

Table 2. Sensitivity, specificity, and predictive values of eFASH signs for diagnosing extrapulmonary tuberculosis in 296 participants.

Variable[a]	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Pleural effusion	54.7% (44.2-65.0)	57.2 (50.1-64.2)	37.7 (29.6-46.3)	72.8 (65.1-79.6)
Pericardial effusion	16.8 (9.94-25.9)	92.5 (88-95.8)	51.6 (33.1-69.8)	70.2 (64.3-75.6)
Splenic hypo-echogenic lesions	11.6 (5.92-19.8)	97.5 (94.3-99.2)	68.8 (41.3-89)	70 (64.3-75.3)
Hepatic hypo-echogenic lesions	7.4 (3.01-14.6)	99.5 (94.3-99.2)	87.5 (47.3-99.7)	69.4 (63.8-74.7)
Ascites	27.4 (18.7- 37.5)	79.6 (73.4-84.9)	38.8 (27.1-51.5)	69.9 (63.5-75.7)
Abdominal lymphadenopathy	27.4 (18.7-37.5)	89.6 (84.5-93.4)	55.3 (40.1-69.8)	72.3 (66.3-77.8)
Pulmonary B lines with subpleural granular artefacts	35.8 (26.2-46.3)	66.7 (59.7-73.1)	33.7 (24.6-43.8)	68.7 (61.7-75.2)
Positive eFASH	93.7 (86.8-97.6)	37.8 (31.1-44.9)	41.6 (34.9-48.5)	92.7 (84.8-97.3)

The sensitivity, specificity, negative and positive predictive value of eFASH signs were compared against the reference standard of definite EPTB. Definite EPTB was defined as the presence of positive microbiology, histology, cytology, culture, or ADA (≥40 U/mL in pleural fluid, or ≥35 U/mL in ascites and pericardial fluid) in any extrapulmonary sample (Supplementary Table 2).
[a]95 with microbiologically confirmed EPTB and 201 with no microbiologically confirmed EPTB (111 probable EPTB, 90 no EPTB). *Abbreviatons:* CI confidence interval; PPV positive predictive value; NPV negative predictive value.

Evolution of eFASH signs

Among the 21 participants with definite EPTB and at least one positive eFASH sign at baseline and follow-up sonographies at 2 and 6 months, pleural effusion was the most common finding (n=16, 76%) (Supplementary Table S6). Resolution of all eFASH signs was documented in 9 (38%) and 16 (67%) participants at 2 and 6 months, respectively. Pericardial effusion, ascites, and hypo-echogenic lesions in the spleen and liver were the only signs that had completely resolved at 2 months. Pleural effusion was the only eFASH sign that persisted beyond 6 months (Figure 2 and Supplementary Table S6). It persisted in four participants: two had septated effusion with fibrin strands, the third was HIV-positive with disseminated tuberculosis co-infection, and the fourth participant was later diagnosed with pulmonary cancer and died in the seventh month.

Among 46 participants with definite EPTB and a baseline and 2-month eFASH examination, eFASH signs fully and partially resolved at 2 months in 12 (26%) and 32 (70%) participants, respectively. A majority of participants with full or partial resolution of eFASH signs at two months had favorable outcomes at 6 months (83% [10/12] and 81% [26/32], respectively). Only two participants had no change or worsening of eFASH findings at 2 months, and both had favorable outcomes at 6 months. The number of deaths between participants with full resolution and those with partial resolution of eFASH were similar (Table 3).

Evolution of clinical signs

A 2-month clinical follow-up was available for 43 out of 95 participants with definitive EPTB. Among these participants, 31 (72%) experienced a complete resolution of clinical signs and symptoms, 10 (23%) a partial resolution, and in 2 (5%), worsening or no change was recorded. Favorable outcomes were more common among participants with full resolution of clinical signs and symptoms than among those with partial resolution (n=28, 90% versus n=6, 60%). Three (30%) of the participants with partial resolution of clinical signs and symptoms at 2 months died, whereas no deaths were reported in those with full resolution of clinical signs and symptoms. However, 2 participants were lost to follow-up. The 2 participants with

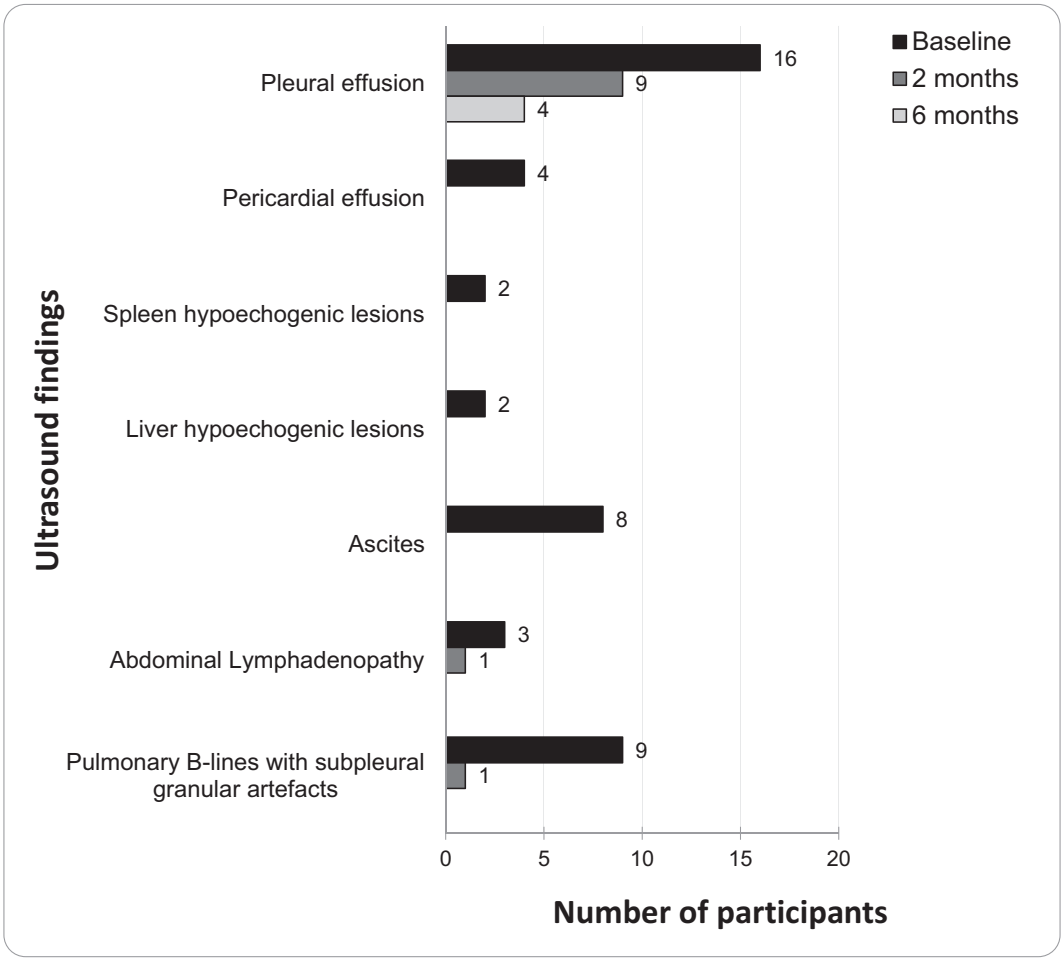

Figure 2. Evolution of eFASH signs over time in 21 out of 28 participants with definite EPTB and a baseline, 2-month, and 6-month eFASH examination. Seven participants did not have any eFASH signs at baseline, whereas 21 had at least one eFASH sign. None of the participants with definite extra-pulmonary tuberculosis had a thickened ileum at baseline. *Abbreviations:* EPTB, extra-pulmonary tuberculosis.

no change in clinical signs or symptoms at 2 months had favorable 6-month outcomes (Table 3).

Discussion

In this analysis of 296 HIV-positive and HIV-negative participants suspected of having EPTB enrolled in a large clinical trial, a positive eFASH had good sensitivity but limited specificity for definite EPTB. Hypo-echogenic lesions in the liver or spleen, pericardial effusion, and abdominal lymphadenopathy had good specificity but limited sensitivity. Clinical signs and symptoms completely resolved 2 months after TB treatment in majority of participants with definite EPTB, whereas sonographic signs were only partially resolved at the same time point. Unfavorable outcomes were observed in a greater proportion of those with partial resolution of clinical signs and symptoms at two months than in those with complete resolution.

To the best of our knowledge, this is the first analysis of the diagnostic accuracy of eFASH signs for extrapulmonary tuberculosis in both HIV-positive and HIV-negative individuals with definite EPTB, while other studies assessed FASH. A study from India that included HIV-positive and -negative individuals with suspected EPTB found that abdominal lymphadenopathy and splenic microabscesses were strongly

Table 3. Resolution of eFASH and clinical signs at 2 months with respect to favorable outcome and death at 6 months.

Signs	Resolution status at 2 months, n (%)		Favorable Outcome, n (%)		Death, n (%)		
			Yes	No	Yes	No	LTFU
eFASH [a]	Fully	12 (26%)	10 (83%)	2 (17%)	1 (8%)	10 (84%)	1 (8%)
	Partially	32 (70%)	26 (81%)	6 (19%)	2 (6%)	28 (88%)	2 (6%)
	No change/ worsening	2 (4%)	2 (100%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)
Clinical [b]	Fully	31 (72%)	28 (90%)	3 (10%)	0 (0%)	29 (94%)	2 (6%)
	Partially	10 (23%)	6 (60%)	4 (40%	3 (30%)	7 (70%)	0 (0%)
	No change / worsening	2 (5%)	2 (100%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)

Complete resolution was defined as disappearance or normalization of eFASH abnormalities; partial resolution as a ≥50% reduction in their extent or severity; and no resolution as persistence, <50% reduction, or worsening. Resolution of clinical signs and symptoms was categorized as fully resolved, partially resolved, or unchanged/worsened based on clinicians' assessment. [a] Forty-six participants with definite EPTB had a baseline and 2-month eFASH evaluation; [b] Forty-three participants had a clinical follow-up at 2 months. *Abbreviations:* EPTB, extrapulmonary tuberculosis; eFASH, extended focused assessment with sonography for HIV-associated tuberculosis; LTFU, lost to follow-up.

associated with TB. This is in line with our results; however, their study evaluated the association of FASH signs with TB, whereas ours assessed diagnostic accuracy (18). Other studies have evaluated the diagnostic accuracy of abdominal ultrasound or FASH in individuals living with HIV with suspected tuberculosis (7, 8). The good sensitivity but low specificity of positive eFASH for definite EPTB is in line with a recently published study conducted in South Africa (19). We found a higher sensitivity but a similar specificity to the results of a 2019 Cochrane review on the diagnosis of abdominal and disseminated TB in people with HIV (7). The low specificity of positive eFASH can be attributed to some frequent but less specific ultrasound findings, such as pleural effusion or ascites (20, 21). However, the specificity increased if multiple sonographic signs were present, which is in line with the findings of another study from South Africa (8). Specific single ultrasonographic signs were pericardial effusion, hypo-echogenic lesions in the liver and spleen, and abdominal lymphadenopathy confirming findings from other studies (7, 18-20).

So far, no studies have analyzed the evolution of clinical signs and symptoms in response to TB treatment in patients with definite EPTB. The association between clinical symptoms and PTB treatment response has been previously described, with studies showing resolution of constitutional symptoms within the first 2 weeks of treatment, which was attributed to the early bactericidal activity of anti-TB drugs (22, 23). This is consistent with our study findings, where there was complete resolution of clinical signs and symptoms in 72% of the participants 2 months after the initiation of TB treatment. With respect to sonographic findings, pleural effusion was the only sign that persisted beyond 6 months. A retrospective study on the resolution of tuberculous pleural effusion in HIV-negative patients with TB pleuritis receiving anti-TB treatment also found that pleural effusion can persist for longer periods, i.e., beyond 12 months (24). In contrast, a case series including patients co-infected with HIV and TB documented completely resolved pleural effusion at 3 months (12). Possible factors associated with the duration of resolution of effusion are the volume of effusion, whether the pleural effusion is loculated, cigarette smoking and ethnicity (24).

This is the first study to assess the evolution of pulmonary B-lines with subpleural granular artefacts in patients receiving anti-TB treatment. Both ultrasound signs have been associated with the diagnosis of TB in previous studies (19, 25). They have also been associated with multiple other conditions, including chronic interstitial lung disease, pneumonia from any cause, and lung malignancies (26), which is in line

with the low sensitivity and fair specificity for definite EPTB in our study.

Our study has several limitations. First, the numbers of participants with two follow-up eFASH examinations, and with unfavorable outcomes were too small for statistical analysis. Second, because eFASH examinations were not consistently performed by the same study physician, some degree of inter-observer variability cannot be excluded; nevertheless, inter-rater agreement was strong (Cohen's $\kappa = 0.84$). Third, we had inadequate information on the specific signs and symptoms of EPTB during follow-up and therefore failed to identify the specific signs that resolved and which ones remained. Finally, participants were included in a TB endemic setting, and the results might not be generalizable to regions where TB is less endemic. The strengths of this analysis are the inclusion of both HIV-positive and HIV-negative participants and the use of a reference standard on the basis of microbiological confirmation of TB in extrapulmonary samples, which has mostly not been done in other studies (19, 27).

Conclusion

A positive eFASH examination demonstrated good sensitivity but limited specificity for definite EPTB, suggesting its utility as a screening tool while requiring confirmatory diagnostic testing. Specificity increased with the accumulation of multiple eFASH findings. The presence of hypo-echogenic lesions in the liver or spleen, pericardial effusion, or abdominal lymphadenopathy was relatively rare, but if present, the lesions were specific for definite EPTB and resolved within 2 months of anti-TB treatment. These findings support the use of eFASH as an adjunct to clinical assessment and confirmatory testing, including for monitoring treatment response.

List of abbreviations:

EPTB Extrapulmonary Tuberculosis
TB Tuberculosis
HIV Human Immunodeficiency Virus
FASH Focused Assessment for HIV-Associated Tuberculosis
eFASH extended Focused Assessment for HIV-Associated Tuberculosis

Ethics Approval and Consent to Participate: The main trial was registered under the Pan African Clinical Trials registry (PACTR201712002829221), and approved by the board of the Ethikkomission der Nordwest und Zentralschweiz (EKBB, Project ID 2017– 02220), Switzerland, the Ifakara Health Institute, Tanzania (IHI/IRB/No010– 2018), and the ethics committee of the National Institute for medical research (NIMR), Tanzania ((NIMR/HQ/R.8a/Vol IX/2897)[14]. Informed consent was obtained from the participants prior to enrollment in the trial. No additional ethical approval was required for this secondary analysis.

Consent for Publication: Permission to publish was obtained from the Director General of the National Institute of Medical Research (Ref No. BD.242/437/01C/85) .

Availability of Data and Materials: The dataset supporting the conclusions of this article is available in the Zenodo repository(https://doi.org/10.5281/zenodo.6697574), and can be accessed upon request.

Trial Registration: The main trial was registered under PACTR, Registration number:PACTR201712002829221, registered December 1st, 2017.

Competing Interests: The authors declare that they have no competing interests.

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