Focused ultrasound to diagnose HIV-associated tuberculosis (FASH) in the extremely resource-limited setting of South Sudan: a cross-sectional study

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ABSTRACT

Objective Our cross-sectional study aimed at evaluating the diagnostic performance of Focused Assessment with Sonography for HIV-associated tuberculosis (FASH) to detect extrapulmonary tuberculosis in extremely resource-limited settings, with visceral leishmaniasis as a differential diagnosis with overlapping sonographic feature.

Design Cross-sectional study.

Setting Voluntary Counselling and Testing Centre (VCT) of Yirol Hospital, South Sudan.

Participants From May to November 2017, 252 HIV-positive patients out of 624 newly admitted to VCT Centre were registered for antiretroviral treatment. According to the number of trained doctors available to practise ultrasound (US) scan, a sample of 100 patients were screened using the FASH protocol.

Interventions Following a full clinical examination, each patient was scanned with a portable US scanner in six different positions for pleural, pericardial, ascitic effusion, abdominal lymphadenopathy and hepatic/splenic microabscesses, according to the FASH protocol. A k39 antigen test for visceral leishmaniasis was also performed on patients with lymphadenopathy and/or splenomegaly. All demographic, clinical and HIV data, as well as FASH results and therapy adjustments, were recorded following the examination.

Results The FASH protocol allowed the detection of pathological US findings suggestive of tuberculosis in 27 out of the 100 patients tested. Overall, FASH results supported tuberculosis treatment indication for 16 of 21 patients, with the treatment being based exclusively on FASH findings in half of them (8 patients). The group of FASH-positive patients had a significantly higher proportion of patients with CD4 count below 0.2 x 10^9/L (n=22, 81%) as compared with FASH-negative patients (n=35, 48%) (p=0.003). Moreover, 48% (n=13) of FASH-positive patients had CD4 below 100 cells/mm^3. All patients tested had a negative result on k39 antigen test.

Conclusion FASH was found to be a relevant diagnostic tool to detect signs of tuberculosis. Further research is needed to better define a patient profile suitable for investigation and also considering diagnostic accuracy.

BACKGROUND

In sub-Saharan Africa, the concomitant high burden of both HIV and tuberculosis (TB) has led to an increasing incidence of extrapulmonary tuberculosis (EPTB).1

EPTB is found more frequently in HIV-positive than in HIV-negative individuals, and the diagnosis is often challenging even in high-income countries due to the low sensitivity and specificity of many available diagnostic tests and broad differential diagnoses.2,3

Ultrasound (US) can aid in the diagnosis of a variety of infectious diseases including EPTB, and the point-of-care ultrasound (POCUS) application is useful especially in resource-limited settings.4,6

Focused Assessment with Sonography for HIV-associated tuberculosis (FASH) is a US protocol aimed to detect sonographic signs of EPTB in patients with HIV infection.7 The examination is focused to find effusions (pericardial, pleural, peritoneal), intra-abdominal lymph nodes enlargement and microabscesses, especially in the spleen and

Strengths and limitations of this study

► Our study analysed the yield of the diagnostic ultrasound FASH (Focused Assessment with Sonography for HIV-associated tuberculosis) protocol in detecting extrapulmonary tuberculosis in HIV-positive patients from an extremely resource-limited setting of rural African, without any other diagnostic opportunities.

► It is worth mentioning that South Sudan is one of the poorest countries in Africa, with a huge need to address health and to meet healthcare demand.

► All ultrasound examinations were performed by a single clinician trained in using ultrasound.

► This is a single-centre experience with a relatively small number of patients recruited.

► Unavailability of a definitive microbiological diagnosis and lack of follow-up were the main limitations of the study.
also in the liver, using six standard scanning planes in the abdomen and lower thorax (figure 1).7

POCUS plays a fundamental role in the diagnosis of different infectious conditions in contexts with limited resources, for example, TB, echinococcosis, amoebic liver abscess, intestinal schistosomiasis and visceral leishmaniasis.4 In particular, within the context of POCUS, FASH protocol was demonstrated to be very useful in the diagnosis of TB in both HIV-positive and HIV-negative subjects, including children and adults.8–10

Currently, in low-income countries with limited infrastructure and weak health systems, especially in terms of qualified human resources, treatment for EPTB is often started based exclusively on a clinical case definition.11 12 This is especially true for South Sudan, the youngest country in the world which has been suffering from years of civil war, economic downturn and famine. Endemic seasonal diseases such as malaria and a recent cholera outbreak (June 2016–February 2018)13 affect South Sudan’s people and further strain the already weak health system.

Our study aimed to (1) evaluate the diagnostic role of FASH for EPTB in an extremely low-resource setting as Yirol Hospital, South Sudan; and (2) provide information on visceral leishmaniasis as potential relevant differential diagnosis with similar sonographic features so requiring a different management in the same context.

**METHODS**

**Study setting and design**

An observational, cross-sectional study was designed and implemented at the Voluntary Counselling and Testing Centre (VCT) of Yirol Hospital in Eastern Lakes State, South Sudan. Yirol Hospital serves as a referral institution for the entire Eastern Lakes State, which has an estimated 442 000 inhabitants.14 Yirol Hospital has no X-ray services available and no health facilities capable of X-ray exams within reasonable distance. The VCT Centre tests patients for HIV infection in accordance with the regulations and guidelines of the Ministry of Health of South Sudan and is authorised to provide antiretroviral treatment (ART) both for inpatients and outpatients. Subjects referred to VCT for counselling and testing are quite diversified and can be categorised into the following: (1) admitted to hospital wards (mainly medical) and sent to the centre for clinical reasons by doctors or clinical officers; (2) sent to VCT by clinical officers of the outpatient department of the hospital; (3) spontaneously coming to the centre for testing; and (4) under TB treatment and also tested positive for HIV (in South Sudan, the treatment of TB is managed by a specific non-governmental organisation that is not integrated in the hospital), or relatives to patients already enrolled.

From May to November 2017, 624 new adult patients accessed the VCT Centre of Yirol Hospital. Among these patients, 252 (40%) were positive for HIV according to the clinical guidelines used in South Sudan (Determine + Unigold).15 These patients were prospectively approached for consecutive enrolment in the study according with the availability of doctors trained for the US scan.

Following informed consent, each patient enrolled underwent a careful history and physical examination (vital signs, weight, height, and diastolic and systolic blood pressure, along with chest, abdomen and lymph node examination). Clinical staging according to the WHO classification (clinical guidelines of South Sudan for HIV) was done before US scan using clinical data only, following the protocol for all patients registered at VCT. Clinical symptoms suggestive of TB (cough, weight loss, haemoptysis, night sweats, fatigue) were enquired, and information on previous TB treatment, isoniazid preventive therapy and ART history was recorded. Acid-fast bacilli sputum smears were carried out when necessary and feasible (presence of chronic cough with sputum), while CD4 counts were done for all patients (PIMA Analyser, Alere, Jena, Germany). For patients with lymphadenopathy (presence of lymph nodes with at least a long axis diameter of more than 1.5 cm) and/or splenomegaly (bipolar axis >13 cm), k39 antigen test, a rapid test for visceral leishmaniasis, was performed (Kala-Azar test, Oscar Medicare, New Delhi, India).

At completion of the clinical examination, sonographic scans were performed according to the FASH protocol.7 In brief, each patient was scanned in six different positions (figure 1) using a portable black-and-white US scanner with convex (3.5 MHz) probe and linear (7 MHz) probe (May–October: DP-30, Mindray; November: M7 Premium, Mindray, Nanshan Shenzhen, People’s Republic of China). All US examinations were performed by a single clinician trained in US. At the end of each examination, the clinician subjectively rated the quality of the US views as ‘excellent’, ‘satisfactory’ or ‘problematic’. All demographic, clinical and HIV data, as well as FASH results and therapy adjustments, were recorded following the examination.
According to FASH protocol, the examination was considered positive (FASH+) if at least one of the following US abnormalities was detected:

- Pericardial effusion (figure 2A).
- Periportal/para-aortic lymph nodes (>1.5 cm in diameter) (figure 2B).
- Focal splenic lesions (figure 2C).
- Pleural effusion or consolidation of the lung (figure 2D).
- Ascites without alternative explanation.
- Focal liver lesions.

It is important to note that TB treatments in South Sudan are delivered by a network of centres that are not directly related to government hospitals nor with the centres providing ART. It was thus of paramount importance to develop close relationship and trust between TB and ART centres. The TB centre in Yirol was informed about the study and agreed to initiate TB treatment based on US and clinical results.

Statistical analysis
Categorical variables were reported as absolute and relative frequencies (percentages). X² test (with Fisher’s correction as required) was used to compare categorical variables using STATA V.13. All statistical tests were two-tailed and statistical significance was assumed for a p value <0.05.

Patient and public involvement
The research question was born to respond to the health needs of the poorest country in the world. The idea was to apply a transportable, economic method, easily applicable to the HIV and TB service in South Sudan. Patients and doctors at the Yirol Hospital were involved to better understand how to improve the project by sharing their own idea. The results of this study will be disseminated through a final report and other activities within the hospital and surrounding communities.
for lung abnormalities and in 85% (n=23) for abdomen abnormalities.

The pathological findings documented in the 27 FASH-positive patients are summarised in table 3. Of the three patients with ascites, one had an additional pleural effusion and one had splenic microabscesses. With regard to one patient with ascites as the only finding, as no other cause for ascites was detected (normal liver, spleen, etc.).
heart and kidneys) and his CD4 count was very low (28 cells/mm³), also in light of the presence of a clinical wasting syndrome, it was decided to start TB treatment.

TB treatment was initiated in 21 (21%) patients after complete clinical and diagnostic examination (table 4). Overall, FASH results supported TB treatment indication in 76% (n=16) of all patients started; of interest, in half of them (n=8), treatment was based exclusively on FASH findings.

Out of 27 patients with abnormal FASH results, only 18 started TB treatment. Several reasons determined this clinical decision: two patients with pericardial effusion left the hospital before starting treatment; five were positive for pleural effusion or lung consolidation and were first treated as pneumonia with improvement; one showed a single focal hypoechoic lesions of the liver with an axis of 2 cm and was addressed to start ART treatment with a strict follow-up of the liver lesion; and one patient with abdominal lymph nodes, peritoneal and pleural-free effusion and splenic hypoechoic lesions died before initiating TB treatment. All patients (n=13) with enlarged lymph nodes, splenomegaly or splenic lesions were tested for antibody against k39 protein and all tests were negative.

The quality of the US examination rated by the clinician was ‘excellent’ in 39% (n=39) and ‘satisfactory’ in 56% (n=56); 5% (n=5) of the exams were considered ‘problematic’.

### Table 2 Additional diagnostic characteristics of the 27 patients with positive results on FASH examination, admitted to Yirol Hospital, South Sudan

| Test                         | Positive | Negative | Not done |
|------------------------------|----------|----------|----------|
| Sputum test                  | 5 (18)   | 13 (48)  | 12 (44)  |
| Kala-Azar test               | 13 (48)  | 14 (52)  |          |
| Symptoms                     |          |          |          |
| Yes                          | 24 (89)  |          |          |
| No                           | 3 (11)   |          |          |
| CD4 (cells/mm³)              |          |          |          |
| <100                         | 13 (48)  |          |          |
| >100 to ≤200                 | 9 (33)   |          |          |
| >200                         | 5 (18)   |          |          |
| Lymph nodes clinical examination |      |          |          |
| Yes                          | 7 (26)   |          |          |
| No                           | 20 (74)  |          |          |
| Lung clinical examination    |          |          |          |
| Positive                     | 3 (11)   |          |          |
| Negative                     | 24 (89)  |          |          |
| Abdomen clinical examination |          |          |          |
| Positive                     | 4 (15)   |          |          |
| Negative                     | 23 (85)  |          |          |

FASH, Focused Assessment with Sonography in HIV-associated tuberculosis.
DISCUSSION

This observational study evaluated the yield of a diagnostic tool (FASH) for EPTB in Eastern Lakes State (Yirol Hospital), South Sudan. The FASH protocol allowed the detection of pathological US findings suggestive of TB in 27 out of the 100 patients tested.

The most frequent pathological findings documented were periportal/para-aortic lymph nodes, pericardial effusion and lung consolidation. FASH findings were more likely to be detected in patients with advanced HIV disease with CD4 count <200 cells/mm$^3$, WHO stage III/IV and low body mass index (BMI). The quality of the US view was mostly rated as excellent and satisfactory by the clinician performing the examination.

Our findings support the important role of this simple, inexpensive and fast technique in resource-limited setting as the Yirol Hospital.\textsuperscript{15-17} Moreover, we were able to identify a core group with the highest yield in FASH, represented by patients with low BMI, low CD4 and advanced WHO stage.

FASH can be taught rapidly to physicians with limited or no prior US experience.\textsuperscript{16} In high prevalence setting, the learning process is facilitated by the presence of pathological findings in a large proportion of HIV/TB coinfected patients,\textsuperscript{19-22} which is supported by our data. Moreover, the fact that many patients under investigation are underweight makes scanning easier, as abdominal volume interfering with scanning becomes less relevant. The examination takes only a few minutes and may provide important findings with a direct and significant impact on patient management.\textsuperscript{2,23} Nevertheless, an important operational question is related to operators who can reliably use this diagnostic technique. In our single-centre experience, we did not succeed in motivating the local clinical officers of the hospital to be trained in the use of US. Young clinicians seemed absolutely more motivated. This is probably related to the poor capacity of the lower cadre of clinical staff in using diagnostic imaging tools and to the fact that the clinical officers in Yirol Hospital are not exclusively dedicated to the care of patients with HIV, and they had many concurrent competing tasks and activities.

Although the diagnosis of EPTB based on abnormal US examination is not certain, it is justified to start a TB treatment based on these findings considering data from the literature and the lack of any other diagnostic tools for further work-up in a rural health setting.

In the region where the study was conducted, there are no epidemiological data for Leishmania spp infection, but this infectious disease is known to be locally prevalent in South Sudan. According to the documents of the Ministry of Health of South Sudan, visceral leishmaniasis is endemic in four states in South Sudan, namely Upper Nile, Unity, Jonglei and Eastern Equatoria; 2.7 million people are considered to be at risk in 28 counties.\textsuperscript{24-26} For this reason, we also tested patients for visceral leishmaniasis as this could be considered a potential differential diagnosis with overlapping imaging findings of enlarged lymph nodes and spleen lesions. With the sensitivity and specificity of the immunochromatographic strip test being more than 90%,\textsuperscript{27} by using the k39 antigen test in patients with an abnormal US result we were able to exclude the presence of visceral leishmaniasis.

Our study has some limitations, which include the relatively small number of patients, the unavailability of a definitive microbiological diagnosis and the lack of follow-up, but we believe our results remain relevant in light of the study setting. South Sudan is a country devastated by civil war, economic downturn and health epidemics such as cholera. As reported by previous studies conducted in such a deprived context with a vulnerable target population affected by HIV-related diseases,\textsuperscript{28,29} healthcare organisational models, human and technical resources, especially the availability of diagnostic tests, may have relevant implications on the pathways of care dedicated to patients with HIV.\textsuperscript{30}

There is an increasing interest in employing US in low-income and middle-income countries given its relatively steep learning curve, ionisation radiation-free nature and its increasing availability at reasonable costs.\textsuperscript{4,17,31} Moreover, it can be portable and can operate with batteries, being independent from a stable electric power supply.\textsuperscript{3} US gel, the only routine supply item needed, can be easily produced locally, thus making US an attractive option in resource-limited settings.\textsuperscript{16} On the other hand, some concern remains about the intrinsic interobserver variability and the potential for diagnostic errors, which should moreover be investigated specifically for TB. While the conditions for a wider implementation are favourable, few studies have been performed in low-income countries especially in rural settings. Our observation adds to the available evidence indicating that the use of FASH for the diagnosis of TB can be useful in this setting.

CONCLUSIONS

FASH was found to be a relevant diagnostic tool to detect signs of EPTB, even in an extremely resource-limited setting such us South Sudan, where HIV and TB incidence is high and radiological and microbiological investigations are scarce. Our data contributed data to better characterise a patient population with the highest yield, namely patients with low BMI, low CD4 and advanced WHO stage, while further research that considers feasibility and diagnostic accuracy is needed. Other interesting fields requiring further research include the training of health operators and the shifting of the procedure to the local staff.\textsuperscript{30}

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2. Heller T, Wallrauch C, Brunetti E, et al. Changes of FASH ultrasound findings in TB-HIV patients during anti-tuberculosis treatment. Int J Tuberc Lung Dis 2014;18:837–9.

3. Giordani MT, Brunetti E, Binazzi R, et al. Extrapulmonary mycobacterial infections in a cohort of HIV-positive patients: ultrasound experience from Vicenza, Italy. Infection 2013;41:409–14.

4. Bélar A, University of Palermo, Palermo, Italy

5. Heller T, Wallrauch C, Gior dani MT, Brunetti E, Binazzi R, et al. Limited access to drugs and review of focused applications for Sub-Saharan Africa. Int J Tuberc Lung Dis 2013;17:342–4.

6. Bélar A, University of Palermo, Palermo, Italy

7. Heller T, Wallrauch C, Gibrilsh C, et al. Focused assessment with sonography for HIV-associated tuberculosis (FASH): a short protocol and a pictorial review. Crit Ultrasound J 2012;4:21.

8. Weber SF, Saravu K, Heller T, et al. Point-of-care ultrasound for extrapulmonary tuberculosis in India: A prospective cohort study in HIV-positive and HIV-negative presumptive tuberculosis patients. Am J Trop Med Hyg 2018;98:266–73.

9. Bélard S, Heuvelsens C, Banderer E, et al. Utility of point-of-care ultrasound in children with pulmonary tuberculosis. Pediatr Infect Dis J 2018;37:636–42.

10. Pool KL, Heuvelsens C, Bélard S, et al. Technical aspects of mediastinal ultrasound for pediatric pulmonary tuberculosis. Pediatr Radiol 2017;47:1839–48.

11. Heller T, Gibrilsh C, Bahlas S, et al. Diagnostic value of FASH ultrasound and chest X-ray in HIV-co-infected patients with abdominal tuberculosis. Int J Tuberc Lung Dis 2013;17:342–4.

12. World Health Organization. Global tuberculosis report. WHO report 2017. WHO/HTM/TB/2017.11. Geneva, Switzerland: WHO, 2017.

13. Republic of South Sudan (RSS), Ministry of Health. Policy and Guidelines for Tuberculosis Control Programme in Southern Sudan, 2016.

14. World Health Organization. South Sudan fact sheet. Geneva: WHO, 2018.

15. Moore CL, Copel JA. Point-of-care ultrasonography. N Engl J Med 2011;364:749–57.

16. Republic of South Sudan (RSS), Ministry of Health. Guidelines for diagnosis, treatment and prevention of visceral leishmaniasis in South Sudan, 2012.

17. Heller T, Wallrauch C, Lessells RJ, et al. Short course for focused assessment with sonography for human immunodeficiency virus/ tuberculosis: preliminary results in a rural setting in South Africa with high prevalence of human immunodeficiency virus and tuberculosis. Am J Trop Med Hyg 2010;82:515–22.

18. van Hoving DJ, Lamprécht HH, Stander M, et al. Adequacy of the emergency point-of-care ultrasound core curriculum for the local burden of disease in South Africa. Emerg Med J 2013;30:312–5.

19. Carrara E, Brunetti E, Di Matteo A, et al. Tubercular liver abscess: an uncommon presentation of disseminated tuberculosis. Infection 2015;43:237–40.

20. van Hoving DJ, van der Meul SC, van der Hoeven SA, et al. Changes of FASH ultrasound findings in TB-HIV patients during anti-tuberculosis treatment. Int J Tuberc Lung Dis 2014;18:837–9.

21. Di Gennaro F, Pisani L, Veronese N, et al. Potential diagnostic properties of chest ultrasound in thoracic tuberculosis—a systematic review. Int J Environ Res Public Health 2018;15:2235.

22. Heller T, Wallrauch C, Gibrilsh C, et al. Focused assessment with sonography for HIV-associated tuberculosis (FASH): a short protocol and a pictorial review. Crit Ultrasound J 2012;4:21.

23. Weber SF, Saravu K, Heller T, et al. Point-of-care ultrasound for extrapulmonary tuberculosis in India: A prospective cohort study in HIV-positive and HIV-negative presumptive tuberculosis patients. Am J Trop Med Hyg 2018;98:266–73.

24. Bélard S, Heuvelsens C, Banderer E, et al. Utility of point-of-care ultrasound in children with pulmonary tuberculosis. Pediatr Infect Dis J 2018;37:636–42.

25. Pool KL, Heuvelsens C, Bélard S, et al. Technical aspects of mediastinal ultrasound for pediatric pulmonary tuberculosis. Pediatr Radiol 2017;47:1839–48.

26. Heller T, Gibrilsh C, Bahlas S, et al. Diagnostic value of FASH ultrasound and chest X-ray in HIV-co-infected patients with abdominal tuberculosis. Int J Tuberc Lung Dis 2013;17:342–4.

27. World Health Organization. Global tuberculosis report. WHO report 2017. WHO/HTM/TB/2017.11. Geneva, Switzerland: WHO, 2017.

28. Republic of South Sudan (RSS), Ministry of Health. Policy and Guidelines for Tuberculosis Control Programme in Southern Sudan, 2016.

29. World Health Organization. South Sudan fact sheet. Geneva: WHO, 2018.

30. Moore CL, Copel JA. Point-of-care ultrasonography. N Engl J Med 2011;364:749–57.

31. Republic of South Sudan (RSS), Ministry of Health. Guidelines for diagnosis, treatment and prevention of visceral leishmaniasis in South Sudan, 2012.

32. National Bureau of Census. Juba - South Sudan, 2017.

33. World Health Organization – Ministry of Health of South Sudan. Summary of Visceral Leishmaniasis (VL) or Kala azar (KA) Outbreak Situation in South Sudan, 2014.

34. Maia Z, Liro M, Mistro S, et al. Comparative study of k39 Leishmania antigen for serodiagnosis of visceral leishmaniasis: systematic review with meta-analysis. PLoS Negl Trop Dis 2012;6:e1484.

35. Di Gennaro F, Marotta C, Pizzol D, et al. Prevalence and predictors of malaria in human immunodeficiency virus infected patients in beira, mozambique. Int J Environ Res Public Health 2016;15:2032.

36. Pizzol D, Veronese N, Marotta C, et al. Predictors of therapy failure in newly diagnosed pulmonary tuberculosis cases in Beira, Mozambique. BMC Res Notes 2018;11:99.

37. Marotta C, Giaquinto C, Di Gennaro F, et al. Pathways of care for HIV infected children in Beira, Mozambique: pre-post intervention study to assess impact of task shifting. BMC Public Health 2018;18:703.

38. Bélard S, Janssen S, Osbak KK, et al. Limited access to drugs for resistant tuberculosis: a call to action. J Public Health 2015;37:691–3.