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Lung Ultrasound Effectively Detects HIV-Associated Interstitial Pulmonary Disease

Daniel T. Marggrander[1,*], Sinem Koç-Günel[2], Nesrin Tekeli-Camcı[3], Simon Martin[4], Rejane Golbach[5], Timo Wolf[6]

[1] *Faculty of Medicine, J.W. Goethe University, Frankfurt, Germany*
[2] *Internal Medicine I, Department of Respiratory Medicine and Allergology, University Hospital Frankfurt, Germany*
[3] *University Thoracic Centre, Department of Oncological Pneumology, St. Elisabethen Hospital Frankfurt, Germany*
[4] *Institute of Diagnostic and Interventional Radiology, University Hospital Frankfurt, Germany*
[5] *Institute of Biostatistics and Mathematic Modelling, University Hospital Frankfurt, Germany*
[6] *Internal Medicine II, Department of Infectious Diseases, University Hospital Frankfurt, Germany*

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ABSTRACT

Objectives To prospectively evaluate lung ultrasound in comparison with radiography and computed tomography (CT) for detecting HIV-related lung diseases.

Methods Ultrasound examinations in HIV-positive patients were evaluated by three raters; available conventional imaging was evaluated by another rater. Results were compared with each other and the definite diagnosis. Interrater reliability was calculated for each finding.

Results Eighty HIV-positive patients received lung ultrasound examinations; 74 received conventional imaging. The overall sensitivity was 97.5% for CT, 90.7% for ultrasound and 78.1% for radiography. The most common diagnoses were *Pneumocystis jirovecii* pneumonia (21 cases) and bacterial pneumonia (17 cases). The most frequent and sensitive ultrasonographic findings were interstitial abnormalities indicated by B-lines, independent of the aetiology. Interrater reliability was high for interstitial abnormalities (ICC=0.82). The interrater reliability for consolidations and effusion increased during the study (r=0.88 and r=0.37, respectively).

Conclusions Ultrasound is a fast, reliable and sensitive point-of-care tool, particularly in detecting interstitial lung disease, which is common in HIV-associated illness. It does not effectively discriminate between different aetiologies. A longer learning period might be required to reliably identify consolidations and effusions.

Introduction

The lung is the organ most commonly affected by complications in people living with HIV (PLWH) (Benito et al, 2012). The most common AIDS-defining disease is *Pneumocystis jirovecii* pneumonia (PCP), which is the second most frequent infectious lung disease in HIV patients (Benito et al, 2012). Several other infectious and malignant diseases also commonly affect the lung in AIDS (Benito et al, 2012; Unnwehr et al, 2020). Furthermore, PLWH have an increased risk of developing bacterial pneumonia, which is the most frequent pulmonary complication and cause of hospitalisation in HIV patients of all ages, independent of disease stage (Benito et al, 2012; Gray and Zar, 2010; Maddedu et al, 2010).

Recent reports have found that lung ultrasound (LUS) might be a feasible point-of-care imaging technique in HIV-associated lung disease (Heuvelings et al, 2016) and particularly in PCP (Giordani et al., 2018; Japiassu and Bozza, 2012; Limonta et al., 2019;). Distinct image artefacts on LUS suggest interstitial disease (indicated by B-lines) or consolidations (indicated by a tissue-like appearance) with high sensitivity and specificity (Volpicelli et al, 2012). For the medical care of PLWH, this could be an opportunity for a widely available bedside imaging technique that does not expose patients to radiation. Furthermore, conventional diagnostic procedures are time-consuming when compared to LUS (Seyedhosseini et al, 2017). Especially in PCP, survival decreases

* Correspondence to: Daniel T. Marggrander, Faculty of Medicine, J.W. Goethe University, Theodor-Stern-Kai 7, H33C (Infectious Diseases), 60590 Frankfurt, Germany

E-mail address: Daniel.Marggrander@kgu.de (D.T. Marggrander).

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with the postponement of therapy (Roux et al, 2014). This suggests that treatment should start without delay until the final, mostly microbiological confirmation, implying the need for a quick diagnostic procedure to detect pulmonary disease.

Given that the abovementioned studies were mostly case series or of retrospective study design, this prospective study systematically evaluated pleural and pulmonary sonography in order to determine the diagnostic accuracy of ultrasound imaging in HIV-associated pulmonary disease.

Methods

From September 2017 to April 2019, HIV-positive patients hospitalised in the department of infectious diseases of the University Hospital Frankfurt, Germany, were enrolled. Inclusion criteria were: confirmed HIV infection, written consent to participate in the study, and definite diagnosis (or exclusion) of a pulmonary disease. Exclusion criteria were: negative or unknown HIV status, consent not given or withdrawn, and lack or refusal of diagnostic interventions necessary for diagnosis (or exclusion) of a pulmonary diagnosis. All hospitalised patients in this time frame were evaluated for participation when they met the inclusion criteria. An attending physician (TW), who could be aware of their diagnoses, included all patients into the study. The institutional ethics committee approved the study (file no. 358/17).

For patients with previously unknown HIV status, the diagnosis of HIV infection was established after both screening and confirmation tests were positive. HIV-related parameters closest to enrolment and, if available, over time were surveyed. All patients received diagnostics according to international standard of care protocols for HIV/AIDS patients (European AIDS Clinical Society 2020), including imaging, microbiology, serology, spirometry, and bronchoscopy, where indicated. Polymerase chain reaction (PCR) from bronchial lavage samples was used to detect fungal, mycobacterial, and viral pathogens. Direct microscopy and standard cultures were used to detect mycobacterial, bacterial, and fungal pathogens. Malignant disease was diagnosed from bronchoscopically- or surgically-acquired samples.

Lung ultrasound was performed in patients who either already received conventional imaging or were scheduled to receive timely imaging. One examiner (DTM) who was blinded to the medical histories performed the examinations, using an APLIO 300 TUS ultrasound system (Toshiba, Tokyo) with a convex 3.5 MHz probe or a linear 10 MHz probe. Two anterior regions in the medioclavicular line ($2^{nd}/3^{rd}$ and $4^{th}/5^{th}$ intercostal spaces) as well as a posterolateral region (posterior axillary line above the diaphragm) were surveyed per hemithorax by longitudinal scans (with the probes' index facing cranially). This protocol, similar to the BLUE-protocol (Lichtenstein DA, 2010), is reproducible according to anatomical landmarks in all participants, including those who are immobilised. The ultrasonographic recordings were evaluated by two additional examiners (SKG and NTC), who were blinded to the diagnoses. All three examiners noted the appearance of B-lines, consolidations, and pleural effusions in each of these regions. A consensus of at least two out of three ultrasound examiners established the ultrasonographic finding. All examiners had at least several weeks of experience in pulmonary ultrasound imaging prior to the study and were not involved in patient care.

Conventional thoracic images, such as chest X-ray (CXR) in anterior and lateral projections or computed tomography (CT), were obtained as clinically indicated. The primary radiological diagnosis was performed as part of the clinical routine. For better comparison within this study, a radiologist (SM) re-evaluated the radiographic images, blinded to the patients, noting the appearance and distribution of interstitial pathologies, consolidations, and pleural effusion. The blinded evaluation was used for comparison to LUS. Sensitivity and specificity of LUS and CXR compared to CT were examined and all were compared to the definite diagnosis. Interrater reliability (IRR) between ultrasound raters was calculated using Light's kappa (κ) on nominal variables and a two-sided, average-weighted intraclass correlation (ICC) model on ratio variables. The χ^2 test with Yates' continuity correction or Fisher's exact test were used for comparing categorical variables. Continuous, not normally distributed variables were analysed using the Wilcoxon-Mann-Whitney test. Linear relationships between variables were assessed using Pearson's r or Spearman's ρ, where appropriate. Normality was tested using the Shapiro-Wilk test. To account for family-wise error in multiple hypothesis testing, p-values were adjusted using the Bonferroni correction. GNU *R* 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria), including the packages *dplyr*, *irr* and *ggplot2*, was used for data analysis (R Core Team, 2020).

Results

Between October 2017 and April 2019, 88 HIV-positive patients were screened for enrolment. Five patients did not provide consent and three were unable to consent; lastly, 80 patients were enrolled. Median age was 47 years (IQR 40 – 52.25), 64 were male, 16 identified as female, two of which were transgender. Median CD4 cell count at enrolment was 137.5/µL (IQR 37.75 – 330.75), median viral load (VL) was 15115.5 IU/mL (IQR 20 – 248250). Thirty-five patients presented with their first manifestation of AIDS during this study, including 27 who were previously unaware of being infected with HIV. Patients who presented with any AIDS-defining disease had, compared to those without AIDS, lower median CD4 cell counts (45/µL, IQR 30.5 – 139.5 vs. 292/µL, IQR 126 – 638; $p<0.001$) and higher median viral loads (170000 IU/mL, IQR 28515.5 – 795500 vs. 21 IU/mL, IQR 20 – 29700; $p<0.001$). See Table 1 for detailed baseline characteristics.

Pulmonary or pleural disease during enrolment was detected in 54 patients. After thorough imaging and laboratory testing, pulmonary disease was excluded in 26 patients. The most common lung diseases were PCP (21 cases) and bacterial pneumonia (19 cases). Six patients presented with cytomegalovirus (CMV) pneumonia, three had non-tuberculous mycobacterial (NTM) infection of the lungs, two presented with tuberculosis (TBC) and two patients had influenza. Furthermore, two cases of pulmonary Kaposi sarcoma (KS), two cases of non-small cell lung cancer (NSCLC) and one case of non-Hodgkin lymphoma (NHL) were seen. Four patients presented with pleural effusion due to extrathoracic disease: two in cases of pancreatitis, one due to acute kidney injury and one secondary to hepatic cirrhosis. Ten patients reported previous pulmonary conditions prior to their hospitalisation: COPD (four cases) and emphysema (three cases), asthmatic disease (one case), history of pleurodesis (one case) and pulmonary arterial hypertension (one case).

Multiple pulmonary diseases coincided in ten patients, nine of which included PCP with either CMV (four cases), mycobacterial infection (three cases), bacterial pneumonia (two cases), or pulmonary embolism (one case). One patient had bacterial pneumonia and NTM infection. When multiple conditions coincided, the patients were analysed according to the disease suspected to be the leading cause of respiratory symptoms, resulting in deviating group sizes discussed below.

LUS was performed in 80 patients, and radiological imaging was available in 74 patients, including 44 CXRs and 51 chest CTs. The diagnosis was established based on imaging performed in external institutions in six patients who were referred; these were however unavailable for the later, blinded examination within this study. For 23 patients, both CXR and CT were available. Detailed sensitivities and specificities of the imaging modalities, according to the clinical

Table 1
Baseline characteristics, serological parameters, and medical history at (or closest to) the time of LUS examination. IQR: Interquartile range. VL: Viral Load. IU: International Units. ART: Antiretroviral therapy. CRP: Serum C-reactive protein. LDH: Serum Lactate dehydrogenase. Median values of continuous variables are depicted with their corresponding IQR, frequencies of categorical variables as percentage.

				Other Lung Disease (*n*=16)		
	All Patients (*n*=80)	PCP (*n*=21)	Bacterial Pneumonia (*n*=17)	AIDS-defining (*n*=6)	Not AIDS-defining (*n*=10)	No Lung Disease (*n*=26)
Age (IQR) [years]	47 (40-52.25)	45 (40 – 50)	49 (40 – 52)	48 (43.5 – 51.75)	55 (48 – 67.5)	45.5 (36 – 52.5)
Gender m/f/t* [%]	80/17.5/2.5	80.9/14.3/4.8	70.6/29.4/0	83.3/0/16.7	70/30/0	88.5/11.5/0
HIV-VL (IQR) [IU/mL]	15115.5 (20 - 248250)	264000 (125000 – 1060000)	27 (20 – 33200)	215000 (46805.5 – 350250)	20 (20 – 22.25)	118.5 (20 – 125675)
HIV-VL Peak (IQR) [IU/mL]	222000 (83950 – 575750)	361000 (148000 – 1070000)	288000 (84200 – 1030000)	301500 (201000 – 379500)	23507.5 (73 – 100150)	172500 (76675 – 465312.8)
CD4 Cell Count (IQR) [1/µL]	137.5 (37.75 – 330.75)	36 (18 – 47)	220 (91 – 484)	95 (77.25 – 154)	233 (102.75 – 631.25)	200 (129.25 – 560.25)
CD4 Cell Count Nadir (IQR) [1/µL]	75 (29 – 169)	35 (16 – 44)	73 (22 – 292)	95 (72 – 154)	151 (86 – 165.5)	149 (63.75 – 200.25)
Years since HIV diagnosis (IQR)	4.5 (0 - 12.75)	0 (0 – 4)	9.5 (4.5 – 28.25)	0 (0 – 1.5)	9 (7 – 16)	6.5 (0 – 11.75)
Prior AIDS-defining disease [%]	28.4	23.8	41.2	16.7	40	23.1
Years since AIDS diagnosis (IQR)	0 (0 – 2)	0 (0 – 0)	4 (0.75 – 21.75)	0 (0 – 1.5)	1 (0.5 – 1.5)	0 (0 – 1.25)
ART at the time of LUS [%]	62.5	33.3	76.5	50	80	73.1
CRP (IQR) [mg/dL]	2.17 (0.58 – 5.46)	2.18 (0.05 – 6.06)	4.02 (1.28 – 12.85)	1.2 (0.66 – 6.43)	2.4 (1.74 – 3.97)	0.84 (0.41 – 2.27)
LDH (IQR) [IU/L]	241 (194.75 – 291.5)	267 (217 – 299)	278 (192 – 306)	214.5 (199.75 – 322.25)	248.5 (222.25 – 367.5)	211.5 (167.75 – 260.5)
Fever [%]	56.25	61.9	76.5	33.3	60	42.3
Shortness of Breath [%]	66.25	85.7	88.24	50	80	34.62
Cough [%]	66.25	76.2	70.6	66.7	70	53.85
Positive Smoking History [%]	71.25	47.62	88.24	83.3	90	69.23
Inhalational Drug Use [%]	7.5	0	11.8	16.7	10	7.7

Abbreviations: IQR, interquartile range; VL, viral load; IU, international units; ART, antiretroviral therapy; CRP, serum C-reactive protein; LDH, serum lactate dehydrogenase
Median values of continuous variables are depicted with their corresponding IQR, frequencies of categorical variables as percentage.
* All transgender (t) patients in our cohort had male-to-female gender reassignment.

diagnoses, are listed in Table 2. LUS examinations took a median of 11 minutes and 48 seconds (IQR 10:09 – 14:19). Examination time decreased with the number of patients seen (see Figure 1a). Median interval between LUS and CT was 3 days (IQR 1.25 – 8), median interval between LUS and CXR was 3 days (IQR 1 – 5.25).

A median one (IQR 0 – 3) out of the six areas scanned per patient showed >2 B-lines (see Figure 2), frequently in PCP, bacterial pneumonia and other lung diseases (see Table 2 and Table 3). The number of views showing >2 B-lines was higher in PCP (median 3, IQR 1 – 4) than in bacterial pneumonia (median 1, IQR 1 – 3; $p>0.2$ vs. PCP) or other lung diseases (median 2, IQR 1 – 2; $p>0.2$ vs. PCP and vs. bacterial pneumonia). The distribution of B-lines was bilateral in 57% of PCP cases, 42% of patients with bacterial pneumonia ($p>0.2$ vs. PCP) and 44% of those with other lung diseases ($p>0.2$ vs. PCP and vs. bacterial pneumonia). The summarised number of B-lines was higher in PCP (median 13, IQR 7 – 18.5) than in bacterial pneumonia (median 11, IQR 5.5 – 12, $p>0.2$ vs. PCP) or in other lung disease (median 10, IQR 8.75 – 11, $p>0.2$ vs. PCP and vs. bacterial pneumonia). Fewer views showing B-lines (median 0, IQR 0 – 0) were detected in patients without lung disease ($p<0.001$ vs. PCP; $p<0.001$ vs. bacterial pneumonia; $p<0.001$ vs. other lung disease), but were present in 19% of patients without a pulmonary diagnosis. Among controls without lung disease, patients who presented with B-lines had higher median viral loads (95900 IU/mL, IQR 23 – 97700) than those without B-lines (median 53 IU/mL, IQR 20 – 135000, $p>0.2$).

Consolidations (see Figure 3) were detected on LUS in 41% of patients with bacterial pneumonia, 14% of patients with PCP ($p>0.2$ vs. bacterial pneumonia), 31% of patients with other lung diseases ($p>0.2$ vs. PCP and vs. bacterial pneumonia), and 11.5% of patients without lung disease ($p>0.2$ vs. any lung disease). Of three patients with PCP and consolidations, two (66.7% of PCP-related consolidations) had aerograms with a cystic pattern (Figure 3) as proposed by other studies (Giordani et al., 2018; Limonta et al., 2019) while one had consolidations without any air bronchograms. In 12 patients with consolidations of other etiology, LUS detected cystic patterns in two cases (16.7%), while 83.3% had linear or no bronchograms on LUS ($p=0.15$ for the difference between PCP and other aetiology).

LUS detected effusions in 13 patients (16.3%). 14% of patients with PCP, 17% of patients with bacterial pneumonia ($p>0.2$ vs. PCP) and 37.5% of patients with other lung disease ($p>0.2$ vs. PCP and vs. bacterial pneumonia) showed effusions on LUS. All patients with PCP who had pleural effusion on LUS also had other pulmonary diseases. Among 12 patients with only PCP, effusions were never detected ($p>0.2$ vs. bacterial pneumonia, $p=0.14$ vs. other lung diseases). 7.7% of patients without pulmonary disease had some degree of detectable effusion ($p>0.2$ vs. any lung dis-

Table 2
Frequencies (f), sensitivity (Sn), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) for pathological findings on LUS, CXR and CT, according to the underlying lung disease. Note that consolidations and effusions were rare in our cohort and interstitial disease was the most indicative sign of lung disease. For individual LUS findings in lung diseases other than PCP and bacterial pneumonia, please see *Table 3*.

	LUS (n=80)				CXR (n=44)				CT (n=51)				
Lung Disease	≥3 B-Lines	Consolidation	Effusion	Any Abnormality	Lung Disease	Interstitial Abnormality	Consolidation	Effusion	Any Abnormality	Lung Disease	Interstitial Abnormality	Consolidation	Effusion	Any Abnormality
Any Lung Disease (n=54)	n=45 f=83.3%	n=15 f=27.8%	n=11 f=20.4%	n=49 Sn: 90.7% PPV: 84.5%	Any Lung Disease (n=32)	n=16 f=50%	n=9 f=28.1%	n=6 f=18.8%	n=25 Sn: 78.1% PPV: 89.3%	Any Lung Disease (n=40)	n=35 f=87.5%	n=14 f=35%	n=5 f=12.5%	n=39 Sn: 97.5% PPV: 92.9%
PCP (n=21)	n=18 f=85.7%	n=3 f=14.3%	n=2 f=9.5%	n=19 Sn: 90.5% PPV: 67.9%	PCP (n=6)	n=5 f=83.3%	n=0 f=0%	n=0 f=0%	n=5 Sn: 83.3% PPV: 62.5%	PCP (n=20)	n=20 f=100%	n=4 f=20%	n=0 f=0%	n=20 Sn: 100% PPV: 86.96%
Bacterial (n=17)	n=14 f=82.4%	n=7 f=41.2%	n=3 f=17.7%	n=15 Sn: 88.2% PPV: 62.5%	Bacterial (n=13)	n=7 f=53.9%	n=6 f=46.2%	n=1 f=7.7%	n=12 Sn: 92.3% PPV: 80%	Bacterial (n=9)	n=8 f=88.9%	n=5 f=55.6%	n=1 f=11.1%	n=9 Sn: 100% PPV: 75%
Other (n=16)	n=13 f=81.3%	n=5 f=31.3%	n=6 f=37.5%	n=15 Sn: 93.8% PPV: 62.5%	Other (n=13)	n=4 f=30.8%	n=3 f=23.1%	n=5 f=38.5%	n=8 Sn: 61.5% PPV: 72.7%	Other (n=11)	n=7 f=63.6%	n=5 f=45.5%	n=4 f=36.4%	n=10 Sn: 90.9% PPV: 76.9%
No Lung Disease (n=26)	n=5 f=19.24%	n=3 f=11.5%	n=2 f=7.7%	n=9 Sp: 65.4% NPV: 77.3%	No Lung Disease (n=12)	n=2 f=16.7%	n=1 f=8.3%	n=1 f=8.3%	n=3 Sp: 75% NPV: 56.25	No Lung Disease (n=11)	n=3 f=27.2%	n=0	n=0	n=3 Sp: 72.7% NPV: 88.9%

ease). Effusions were small (<5 mm hypoechoic margin) in seven cases and large (>5 mm margin) in six cases; no difference was observed in the side where effusion occurred (three on the right, seven on the left, three bilaterally, p>0.2).

IRR was calculated for the number of B-lines observed per view (mean ICC=0.82, range=0.75 – 0.87; p<0.001), for the presence of consolidations (mean κ=0.12; p>0.2) and pleural effusion (mean κ=0.12; p>0.2) on LUS. IRR for effusion was calculated separately for large effusions (mean κ=0.26) and for small effusions (mean κ=0.03). Since all raters evaluated the sonographic images in the same order, IRR could also be calculated as a function of the patients observed, as depicted in Figure 1b. Linear correlation between the number of patients and corresponding IRR values are r=0.88 for consolidations, r=0.37 for pleural effusion, and r=-0.11 for B-lines.

CT examinations were available in 51 patients, 40 of which had a lung disease. CT detected abnormalities in 42 (82.4%) cases: interstitial abnormalities (reticular markings, nodules or ground glass opacities) in 37 patients (72.6%), consolidations in 14 cases (27.5%), and effusion in five cases (9.8%).

Forty-four CXR examinations were available; 32 in patients who were diagnosed with a pulmonary disease. Any abnormalities were seen in 32 cases (72.7%). Interstitial abnormalities were detected on CXR in 18 instances (41%), consolidations in 10 cases (22.7%) and effusion in seven patients (15.9%).

In 23 patients who had both CXR and CT performed, CXR was 79% sensitive and 100% specific in detecting any abnormality, as compared to CT. CXR was 68.8% sensitive in detecting interstitial disease, 80% sensitive for consolidations and 75% sensitive for effusion, as indicated on CT.

Among 51 patients who had CT and LUS performed, LUS detected any abnormality (as compared to CT) with a sensitivity of 88.1%. B-lines on LUS had, compared to interstitial abnormalities on CT, a sensitivity of 86.5% and a specificity of 71.4%. LUS was 35.7% sensitive and 86.5% specific for detection of consolidations when compared to CT. Effusions were detected on LUS with 60% sensitivity and 95.7% specificity as compared to CT.

Discussion

Interstitial abnormalities were frequent findings on CT and LUS, while CXR detected them less frequently. The detection of views with three or more B-lines (Figure 2) on LUS seemed to be the most indicative finding for lung disease, although less sensitive than interstitial abnormalities on CT. B-lines appeared significantly more frequently and were more numerous in pulmonary disease than in patients without lung disease. However, neither frequency nor number of views with more than three B-lines distinguished between different pathologies: PCP appeared to cause these artefacts more frequently in multiple and bilateral locations; however, the difference to other agents was not significant, and diseases may mimic each other. This diagnostic challenge is also known in conventional imaging, where entities such as bacterial pneumonia in HIV, KS, mycobacterial or CMV-infection also frequently present as multifocal and diffuse interstitial disease (Borie et al, 2013; Busi Rizzi et al, 2008; Gasparetto et al, 2009; Unnewehr et al, 2020). Another study demonstrated that bilateral distribution of B-lines is indicative of PCP, as compared to other lung diseases in PLWH (Japiassu et al, 2012); however, that cohort was recruited from an intensive care unit, with more severe lung diseases than in the population described here. In less critical patients, the distribution in PCP may be more discrete. The current study also detected B-lines in patients without lung disease, resulting in a low specificity for LUS. Although the positive predictive value for B-lines remains high, due to the prevalence of lung diseases in the participants, this might not apply

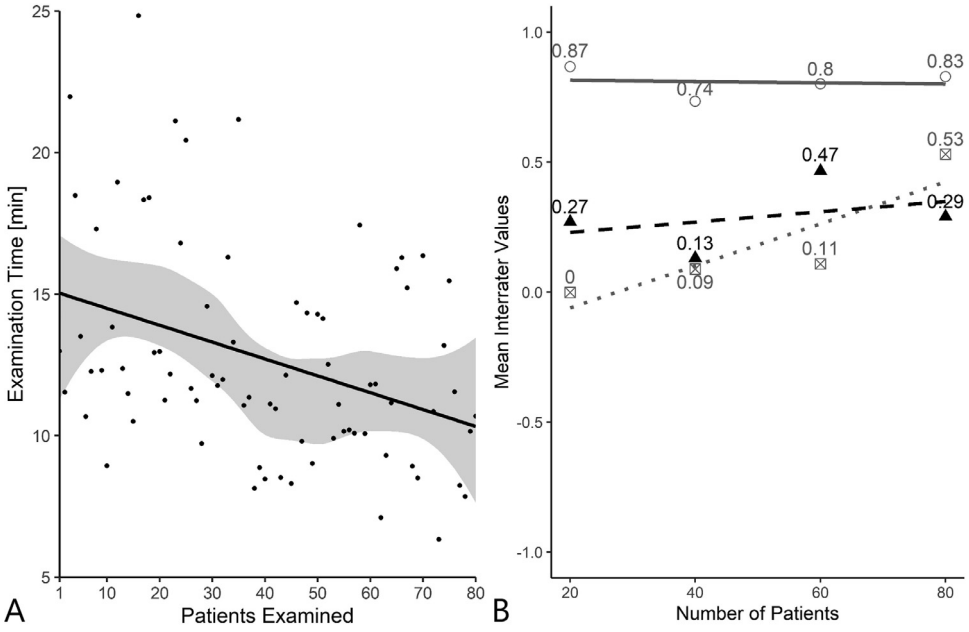

Figure 1. Ultrasound examinations over the course of the study. a) The examination time decreased with the number of enrolled patients, both when assuming linear (solid line: linear regression, ρ=-0.37) and non-linear (grey area: 95% confidence of local regression) models, suggesting a learning effect from routine examinations. b) IRR values for B-lines (solid line and circles, ICC), consolidations (dotted line and squares, Lights' κ) and effusion (dashed line and triangles, Lights' κ) over the course of the study, plotted for each consecutive group of 20 patients. While IRR remains steady for B-lines (r=-0.11), increasing IRR values for detection of consolidations (r=0.88) and effusions (r=0.37) are observed, suggesting the necessity of a longer learning period for the latter two. IRR values for both ICC and Lights' κ can range between 1 (perfect agreement) and -1 (complete disagreement).

Table 3
Individual LUS findings of different diseases (other than PCP and bacterial pneumonia) that we encountered. Although we summarised them as one group for statistical analysis, this table demonstrates the heterogeneity in HIV-associated pulmonary disease detectable by LUS.

Lung Disease	**Frequency of ≥3 B-Lines** [%]	**Frequency of Consolidations** [%]	**Frequency of Effusions** [%]	**Frequency of any Abnormality** [%]
Secondary Effusion (n=4)	50	50	100	100
CMV-Pneumonia[¶] (n=2)	100	0	0	100
Influenza (n=2)	50	0	0	50
COPD/Emphysema (n=2)	100	50	0	100
KS[¶] (n=2)	100	50	0	100
NSCLC (n=2)	100	50	50	100
TBC[¶] (n=1)	100	0	0	100
NHL[¶] (n=1)	100	0	100	100

[¶]AIDS-defining disease.

to settings with a lower prevalence. Patients without lung disease presenting with B-lines had higher median VL than those without B-lines on LUS. Pulmonary interstitial abnormalities, associated with increased VL, are a known phenomenon on CT in otherwise healthy PLWH (Leader et al., 2016). Some models hypothesise chronic inflammatory changes in the lung due to HIV infection (Almodovar, 2014). The patients in the current study were largely enrolled in advanced stages of HIV/AIDS with high VL (see Table 1), which could contribute to this observation. The relationship between sonographic detection of interstitial abnormalities and the corresponding VL did not reach statistical significance in this analysis. This phenomenon should however be borne in mind and the clinical presentation considered when finding interstitial abnormalities, particularly in PLWH. This also implies that the specificity of B-lines may improve in patients receiving effective viral suppression therapy.

Consolidations (Figure 3) occurred more frequently in bacterial pneumonia on LUS, but again the between-group differences were not significant. Two studies suggest that cystic patterns of air artefacts within a consolidation are specific to PCP (Giordani et al, 2018; (Limonta et al. 2019); two-thirds of the consolidations we detected in cases of PCP did indeed show cystic abnormalities. However, this morphology was also seen in patients with lung disease of other aetiology, although less frequently. Due to the overall low frequency of consolidations and the consecutively insignificant differences, the proposed specificity (Giordani et al, 2018) of this finding for PCP could neither be supported nor dismissed. As LUS only detects consolidations that reach the pleural surface, CXR appears more advantageous in comparison. However, it is notable that not all patients had CXR and CT for confirmation performed, while others only received either CXR or CT. In clinical practice, follow-up is often performed using CT

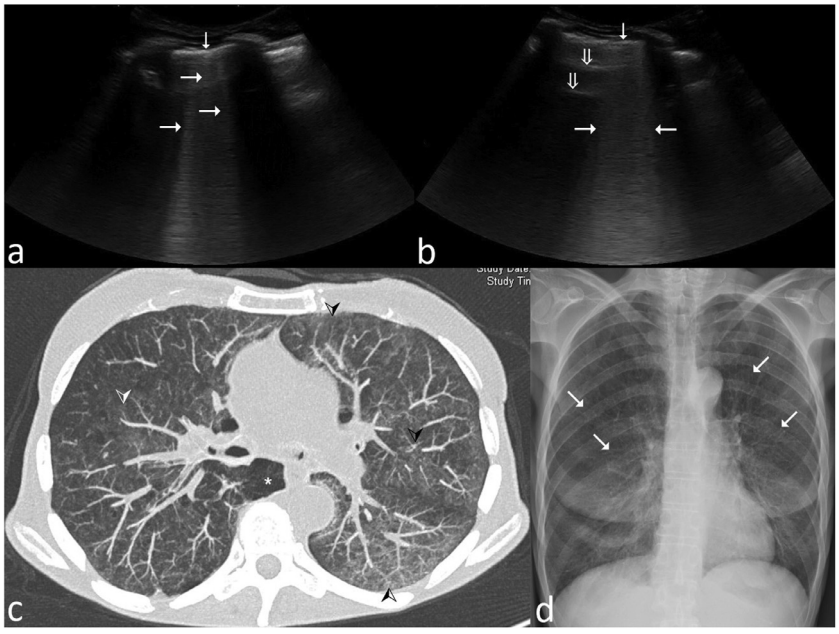

Figure 2. Lung ultrasound (LUS), computed tomography (CT) and chest x-ray (CXR) of a patient with *Pneumocystis jirovecii* pneumonia (PCP). a) and b) B-lines (horizontal arrows) are vertical, hyperechoic artifacts that arise from the pleural line (vertical arrows) and extend to the bottom of the screen. They move with respiratory efforts and obliterate horizontal imaging artifacts, so called A-lines (double arrows) that can usually be seen in the healthy lung and pneumothorax. Three or more (Lung and Interstitial Syndrome, 2010) B-lines per intercostal space correspond to thickening of interlobular septa, associated with edema, infectious disease or fibrosis. Separate B-lines (a) may merge with increasing interlobular thickening and (b), become indistinguishable (referred to as "coalescent"), which corresponds to ground glass opacities on CT (Lung and Interstitial Syndrome, 2010). c) Chest CT showing bilateral reticular markings, ground glass opacities (arrowheads) and emphysema (*). d) CXR showing bilateral perihilar reticular markings (oblique arrows). Note that the focal distribution of abnormalities can be assessed on CT (cross sectional imaging) and LUS (according to probe position), in comparison to the sagittal projection of CXR, which decreases the spatial resolution.

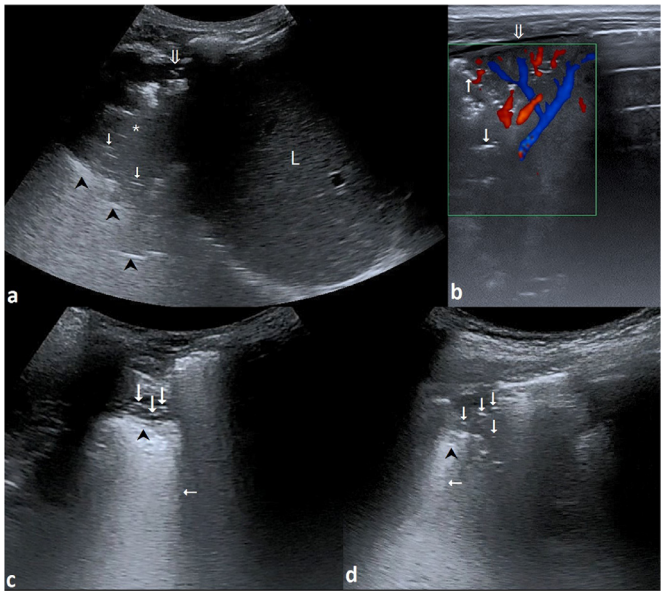

Figure 3. Sonographic features of consolidations.
a) and b) Large consolidation (*) that appears hypoechoic and tissue-like, comparable to the liver (L) parenchyma, with linear bronchograms (vertical arrows) in a patient presenting with tachypnoea and fever after discontinuation of ART. The *shred sign* refers to its irregular, hyperechoic borders (arrowheads) that indicate aerated lung. A small effusion (double arrow) is detectable. Using a linear probe, colour doppler reveals patent arterial (red) and venous (blue) vascularisation reaching the periphery of the lesion, ruling out the differential diagnosis of a pulmonary embolism in that area. The patient had bacterial pneumonia.
c) and d) Small consolidations with *cystic* pattern, i.e. hyperechoic spots within a hypoechoic consolidation that is bordered by the *shred sign* (arrowheads). While the shred sign is accompanied by comet tail artifacts (horizontal arrows), the hyperechoic spots are not, similar to what other studies describe.(Giordani et al., 2018) Image c) was seen in a patient with PCP, while d) was present in a patient with bacterial pneumonia and no current or prior history of PCP.

when CXR is abnormal in the first place (which explains the high specificity when comparing the two), so CXR performed particularly well in this selective subset of patients, but was less effective in the overall analysis (Table 2).

Effusions were seen on LUS among patients with PCP, bacterial pneumonia, other lung disease, and even among those without pulmonary disease. Due to the overall low incidence of this finding, the frequency of effusion among these groups did not significantly differ. However, it should be noted that when only assessing cases of PCP without other coinciding lung disease, no effusions were detected at all. Although not reaching statistical significance, this result corresponds to other studies that showed absence of effusion specifically in PCP (Giordani et al, 2018; Japiassu et al, 2012). Similar to those studies, effusion in cases of PCP indicated another pathology secondary to *Pneumocystis* infection.

Since this study only assessed anterior and lateral regions in the protocol, it left a blind spot in posterior lung areas. Accuracy might increase with capturing more areas of the lung surface (Heuvelings et al, 2016; Volpicelli et al, 2012); another study that assessed 28 thoracic regions showed a frequency of 100% (Giordani et al, 2018) for B-lines in PCP; however, this study was retrospective and had a smaller population.

The comparability of the results was limited since CT and CXR were performed on different subsets of patients, as it would be unethical to expose patients to higher levels of radiation than indicated. Furthermore, the LUS results were consensus findings as opposed to findings on CXR and CT. LUS also assessed limited areas anteriorly and laterally, while CXR and CT scans of the entire lung were analysed; therefore, this study might have underestimated LUS accuracy. Furthermore, the time difference between lung ultrasound and conventional imaging might have influenced the comparison in some patients, when pathologies seen in one modality progressed or regressed in the days before observations

in the other modality were made; however, the delay was modest in this study.

The overall IRR (mean ICC=0.82) for B-lines suggests excellent (Hallgren, 2012) interobserver reliability and therefore an effective diagnostic finding. IRR for consolidations and effusions was overall low (κ=0.12 each), did not reach statistical significance, and suggests slight agreement at best (Hallgren, 2012). IRR remained high for B-lines over the entire course of the study withoutchanging particularly (r=-0.11), while positive relationships between the number of patients and IRR for both consolidations (r=0.88) and effusions (r=0.37) were indicated (see Figure 1b). This suggests that a longer learning period may be required to effectively distinguish the latter two findings; therefore, the current results might have underestimated IRR in these findings. The low IRR for effusions seems especially surprising, given that ultrasound is routinely used to detect effusion. One reason might be the low prevalence of effusions in the current patients. Although larger effusions (>5 mm margin) were more reliably detected, smaller hypoechoic margins (<5 mm) might have been dismissed by some raters as not being pathological. Furthermore, effusions might have been harder to detect on LUS, since scans were performed in the supine position. In comparison, detection of B-lines was more frequent than detection of consolidations and effusions (see Tables 2 and 3) and appears more reliable, even with less LUS experience.

As discussed in previous studies covering lung ultrasound, this modality might be used in remote and resource-poor settings where conventional diagnostics are unavailable (Ellington et al, 2017). In HIV and AIDS this appears particularly promising, given the disease burden in middle- and low-income countries (Shao and Williamson, 2012).

Although this work represents (to our knowledge) the largest controlled study on LUS in HIV-associated lung disease to date, it had several limitations. The group sizes for each individual disease were small. Not all patients received the same radiographic imaging for comparison. The ultrasound protocol itself might have missed pathologies in areas not covered. Although unaware of the diagnoses, the primary examiner could not be blinded to the clinical presentation of the patients. The increase in IRR suggests that some of the raters may have been inexperienced at the beginning of the trial. Larger trials, ideally with patients under effective antiretroviral therapy for control, are needed to verify the results. The strengths of the study were its prospective design and systematic, controlled analysis of LUS, and its IRR in PLWH with and without lung disease.

Conclusion

This study demonstrated that LUS is effective in detecting interstitial HIV-associated lung disease. B-lines appeared to be fast and reproducible imaging results on LUS, with high sensitivity and reliability. Other LUS findings were less frequently observed and seemed to require a longer learning period. The fast availability at the patients' bedside, its sensitivity, and absence of radiation suggest that the use of LUS as a screening tool might aid in deciding which patients need exposure to further diagnostics. This might help spare other patients from irradiation when sonography provides sufficient diagnostic data, and may accelerate diagnostics in critical conditions until reaching a final confirmation.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Contributions

Ultrasound examinations: DTM. Ultrasound review: SKG, NTC. Collection of data: DTM, TW. Radiological imaging review: SM. Data analysis: DTM, RG. Manuscript: DTM. Critical revisions: SKG, NTC, SM, RG, TW. All authors helped finalize the text and approve of the definite manuscript.

Compliance with ethical standards

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