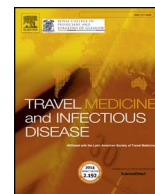




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Pocket-size point-of-care ultrasound in rural Uganda — A unique opportunity “to see”, where no imaging facilities are available

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ABSTRACT

Background: In the developing world, only a small minority of patients have access to radiological services. Over the past decade, technological developments of ultrasound equipment have led to the emergence of point-of-care ultrasonography (POCUS), which is widely used by healthcare professionals of nearly all specialties. We hypothesized that physicians with only basic POCUS training, but with telemedicine support, can use POCUS successfully in rural hospitals in sub-Saharan Africa.

Method: During a 14-day voluntary clinical work session in a rural hospital in central Uganda, bedside ultrasound scans were performed by use of a pocket-size portable machine by a physician who underwent a five-day training period. All the POCUS studies were reviewed by radiologists and cardiologists abroad with the use of telemedicine.

Results: During the study period, 30% of patients received a POCUS-augmented physical examination. 16 out of 23 patients (70%) had positive findings; in 20 of them (87%), the management was changed. The technique was successfully used on trauma casualties, patients suffering from shock, patients with cardiorespiratory symptoms, and patients undergoing invasive procedures.

Conclusions: In a very resource-limited environment, POCUS conducted by basically trained primary care physicians with telemedicine support is a powerful diagnostic tool in a variety of medical conditions.

1. Introduction

The field of medical imaging, stimulated by advances in digital and communication technologies, has revolutionized almost every aspect of medicine over the last decades. This revolution, however, has taken place almost exclusively in medium and high-income countries.

However, over 80% of the world's population live on less than 10\$ (USD) per day and as much as 47% of Africa's population lives on 1\$ or less a day [1,2]. Many rural hospitals in the developing world have no reliable water and electricity supply [3]. As many as fourteen African countries have no radiologists at all, and most have fewer than thirty, with only five residency programs for radiology on average [3,4]. The lack of water and electricity, and of human resources often goes with a

general lack of public infrastructure, such as roads and railway links. As a result, only 220 million people in the developing world (out of more than five billion) have access to basic radiology services, such as X-rays [3].

Over the past decades, ultrasound equipment has become more compact, portable, of higher quality, and less expensive. This has led to the emergence of point-of-care ultrasonography (POCUS), that is, ultrasonography brought to the patient and performed by the treating clinician in real time, allowing findings to be directly correlated with the patient's signs and symptoms [5].

Nowadays, smartphone-size machines retails for less than 10,000\$ have dual probes with low- and high-sound wave frequencies, allowing the operator to observe superficial (vascular, soft tissue) as well as deep

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(cardiac, abdomen) anatomical structures, augmented by color Doppler capabilities [6]. Some machines even include M-mode capabilities [7].

In high-income countries, this diagnostic modality is utilized by healthcare professionals of nearly all specialties [5]. Many medical schools have integrated POCUS into their teaching curriculum [5]. Studies have shown that physicians and others can be expected to perform effective and accurate scans after as little as 3 h of didactic training and 5 h of hands-on training [8,9].

The advantages of ultrasound in resource limited environment are well established. In 1985 the World Health Organization (WHO) concluded that there are “very real advantages to be gained from the use” of ultrasound, and noted its potential for “improved patient management and care of the individual” in developing areas [10]. A recently published, systematic review of the use of portable ultrasound devices in low- and middle-income countries found 36 published articles [7]. However, all but one study were performed in large hospitals with a relatively wide variety of facilities and the help of an experienced sonographer.

POCUS, performed with portable devices, was successfully used by sonographers and cardiologists in low- and middle-income countries in a variety of medical conditions. Kobal et al. successfully evaluated 126 patients referred for cardiac evaluation in rural Mexico [11]. Few studies demonstrated the successful use of hand-held ultrasound to screen for rheumatic heart disease, reduced ejection fraction, valvular regurgitation, wall motion abnormalities and presence of pericardial effusion. Various ultrasonographic protocols were developed for risk stratification of patients with malaria, filariasis and dengue in the developing world [7]. In India and Thailand POCUS was successfully used to confirm the position of an endotracheal tube and assess laryngeal edema before extubation [12,13]. Hand-held ultrasound devices were successfully used by aid teams to perform modified FAST examinations in 2010 earthquake in Haiti. The authors reported that ultrasound examination findings changed the clinical management in 70% of patients evaluated [14]. Similar findings were reported by in Guatemala and China [7]. POCUS was reported to be useful for diagnosis of intussusception, evaluation of soft tissue masses, breast masses and various gynecological conditions [7].

In this article we describe our experience, and review the potential applications of a pocket-size, portable ultrasound scan with telemedicine support in a rural hospital in Uganda.

2. Material and methods

Kiboga hospital is a governmental general hospital with 210 beds. It is located 3 h drive on rugged road northwest of Mulago National Referral Hospital, Kampala, Uganda. The Kiboga campus has no running water, very limited laboratory capabilities (complete blood count, blood smear microscopy, malaria and HIV rapid diagnostic tests, CD4 count and Gene Xpert machine), and has no imaging facilities. The hospital is understaffed, with only one local physician on call at any given moment. This hospital serves a population of more than 250,000.

All ultrasound scans were performed with a pocket-size, portable ultrasound machine (Vscan[®], GE Healthcare, USA) with a single low-frequency probe (1.7–3.8 MHz). The studies were performed over a period of 14 days by an internal medicine resident, who was providing volunteer medical care as part of the Israeli Medicine on the Equator project. All studies were conducted for clinical indications.

Most of the physician's time during this period was spent on clinical work in the hospital and in outreach clinics. Prior to deployment, the resident underwent a five-daylong, formal POCUS course. The course's curriculum is described in [Box 1](#). Course syllabus included: cardiac ultrasound anatomy with practical hands-on sessions, utilization of ultrasound to narrow differential diagnosis of shock, lung ultrasound, focused assessment with sonography for trauma (FAST) as well as hands-on sessions of ultrasound guided vascular access.

All the POCUS studies were reviewed by experienced radiologists

and cardiologists abroad with the use of cellular phones, commercially available video-chat software, and a 3G cellular data network. Most studies were reviewed online and some within 3 h of the procedure. The radiologist was able to visualize both the ultrasound scan and the patient ([Fig. 1](#)). This simple method of telementored ultrasound screening was previously investigated in healthy volunteers and shown to be useful [9].

Ultrasound equipment was donated by Eldan-Life Science & Healthcare Solutions for the length of the mission.

Patients' data were recorded as part of medical history taking. All radiologic tests were performed as part of routine clinical work. An informed consent was therefore not required. There is no formal ethics committee in Kiboga hospital. Therefore, in concordance with ICMMJE recommendations [15], the data publication was discussed with institutional management and found to be in accordance with the Helsinki Declaration as revised in 2013. We believe that publication of data, collected during routine and voluntary clinical work, is in concordance with all international legal, ethical and regulatory norms and standards.

3. Results

Over a 14-day period, 23 of the 75 (30%) acutely ill patients received, by clinical indication, augmented physical examination using pocket size ultrasound machine. 16 of the 23 patients who received POCUS (70%) had positive ultrasonography findings and for 20 of them (87%) management was changed (in four patients, a previous clinical diagnosis was ruled out). These included three trauma patients, seven patients with cardiorespiratory symptoms, and four patients suffering from shock. Three patients were evaluated because of suspected hepatosplenomegaly, two had clinical suspicion of significant pleural effusion and two were confirmed to have urinary retention. The results of POCUS exams and their clinical implementations are summarized in [Table 1](#). As mentioned previously, all the studies were reviewed by experienced radiologists and cardiologists, who were all in agreement with the treating physician.

3.1. Trauma

Focused Assessment with Sonography in Trauma (FAST) was performed on three patients. Two of them suffered blunt trauma, secondary to motor vehicle accidents, and one suffered penetrating abdominal stab wounds. Two of the three studies were positive, demonstrating a large amount of free intraperitoneal fluid ([Fig. 1](#)). Both patients were promptly referred to a tertiary hospital in Kampala after initiation of fluid resuscitation. They were diagnosed with high-grade splenic injuries; one of them was treated conservatively while the second patient underwent urgent splenectomy. The third patient had negative study and was discharged after observation.

3.2. Focused cardiac ultrasound

Echocardiographic studies were performed on seven patients with main symptoms of dyspnea, chest pain, and palpitations.

Severely reduced left ventricle ejection fraction (LVEF) was demonstrated in three patients with no previous history of congestive heart failure. One of these patients, a 40-year-old, previously healthy woman was diagnosed with atrial fibrillation, severe mitral regurgitation and significant mitral stenosis, most probably of rheumatic origin ([Fig. 2](#)). She was started on aspirin and beta-blockers (anticoagulation drugs were not available) and referred to a cardiologist in Kampala.

Large pericardial effusion without echocardiographic signs of tamponade was diagnosed in a previously healthy 24-year-old male with fever, cough, and chest pain ([Fig. 3](#)). Serial echocardiographic studies were performed. No fluid enlargement or signs of chamber wall collapse were recorded.

Box 1

Five days course curriculum review.

- Hands on training on performance of all echocardiographic cardiac views.
- Learning how to differentiate between normal and abnormal echocardiographic exams.
- Learning how to screen for and identify 6 major types of shock: hypovolemic, septic, tamponade, pulmonary embolism, cardiogenic and pneumothorax.
- Performance of chest ultrasound for the diagnosis of pneumothorax, pulmonary edema, lung atelectasis, pleural effusion and pneumonia.
- Performance of the FAST exam for diagnosis of intra-abdominal bleeding in the setting of blunt trauma.
- Understand challenges involved in incorporating focused cardiac ultrasound during cardio-pulmonary resuscitation efforts.

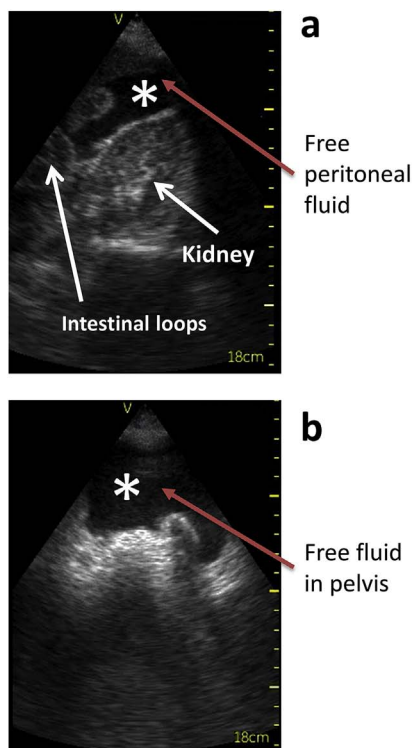
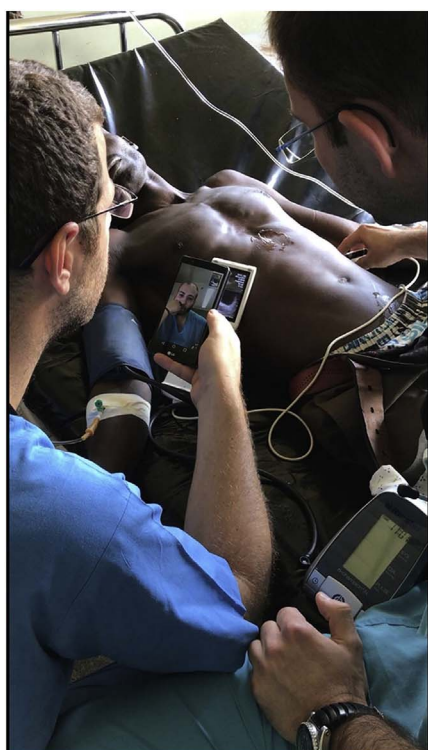


Fig. 1. Focused assessment with sonography for trauma (FAST) performed on 35 years old male admitted 24 h after involvement in motorcycle accident. The study is performed by treating physicians and reviewed by radiologist in Israel. Large amount of free intraperitoneal fluid (*) was demonstrated in right upper quadrant (a) and in suprapubic view (b).

Normal LVEF was demonstrated in three patients, two of whom had been previously diagnosed with systolic heart failure according to their medical history and physical examination. These echocardiographic findings led to additional diagnostic workup in these patients. Consequently, one of them was diagnosed with nephrotic syndrome. All of them were discharged and were in good health during a follow-up meeting one week later.

3.3. Liver and spleen sonography

Suspected liver and spleen enlargement was confirmed in three patients. Ascites was ruled out in all of these studies. No focal findings were demonstrated. One of the patients had previously been wrongly diagnosed as having a severe kidney enlargement, secondary to polycystic kidney disease on clinical ground.

3.4. Lung ultrasound

Suspected pleural effusion was evaluated in two patients with clinical diagnosis of pneumonia. In both studies small unilateral pleural effusion was confirmed, but planned thoracentesis was deferred because the amount of fluid was deemed too small and no characteristics of empyema were observed.

3.5. Guidance of procedures with ultrasonography

POCUS was successfully used for guidance of an improvised suprapubic urine catheter insertion and lumbar puncture (LP) (Fig. 4).

Nine previous attempts to perform LP on a 14-year-old patient with suspected meningitis failed. Using POCUS, under sterile conditions, the intraspinal cavity was found on first needle insertion. Spinal fluid analysis showed no white blood cells and the diagnosis of meningitis was deferred.

The suprapubic catheter was placed in a 24-year-old male who was hospitalized following symptoms of encephalopathy, anuria, and abdominal mass. Urinary retention was confirmed by US scan and presumptive diagnosis of uremic encephalopathy was established. Multiple attempts of urethral catheterization failed. Under US guidance, a single lumen central line catheter was inserted, using Seldinger wire technique, in suprapubic position. Three liters of urine were drained. During the next 48 h a rapid improvement in urinary output and general condition of the patient were recorded. After three days he was referred for urologic consultation in Kampala.

Both procedures were performed without any complications, with online guidance from an experienced urologist and neurologist.

3.6. Additional studies

Ultrasound was used for the evaluation of two patients with

Table 1
The results of 23 POCUS exams and their clinical implementations.

Clinical diagnosis	Imaging findings	Impact on management
Two young males admitted due to blunt abdominal trauma as a result of MVA.	Large amount of free intraperitoneal fluid.	Fluid and blood resuscitation was initiated, and the patients transferred to a tertiary level care hospital.
Young male admitted due to stab wound in left flank.	No intraperitoneal fluid or evidence of pneumothorax was demonstrated.	The patient was discharged home after a short observation.
Young male admitted due to fever, cough, chest pain and dyspnea.	Large pericardial effusion without echocardiographic signs of cardiac tamponade.	Serial echocardiographic studies were performed. No increase in the amount of fluid or signs of chamber wall collapse were observed.
Two elderly patients with new symptoms of general weakness and exertional dyspnea.	Severely reduced LVEF without major valvular abnormalities.	Medical treatment was initiated with good clinical response.
Middle aged woman with history of palpitation and dyspnea.	Severely reduced LVEF, severe MR and significant MS, most probably of rheumatic origin.	Patient started on aspirin and beta-blockers (anticoagulation drugs were not available) and referred to a cardiologist.
Three patients with clinical diagnosis of CHF	Normal LVEF without pericardial effusion or major valvular abnormalities.	Additional diagnostic workup was performed leading to diagnosis of nephrotic syndrome in one of the patients.
Two patients with abdominal distention.	Liver and spleen enlargement was demonstrated. Ascites was ruled out. No focal findings.	Workup for hepatosplenomegaly was initiated.
Young male with clinical diagnosis of PKD	Normal size and structure kidneys. Liver and spleen enlargement.	PKD diagnosis was deferred and workup for hepatosplenomegaly was initiated.
Two children with slowly resolving pneumonia and suspected pleural effusion	Small unilateral pleural effusion was confirmed.	Due to small amount and sonographic parameters of the fluid, planned thoracocentesis was deferred.
Fourteen years old patient with suspected meningitis. Nine previous attempts to perform LP failed.	Using US, the intraspinal cavity was found on first needle insertion.	Spinal fluid analysis was normal; diagnosis of meningitis was deferred.
24-year-old male hospitalized following symptoms of encephalopathy, anuria, and abdominal mass. Multiple attempts of urethral catheterization failed.	Large bladder was demonstrated.	Under US guidance, a single lumen central line catheter was inserted, using Seldinger wire technique, in suprapubic position. Three liters of urine were drained.
Two elderly patients with suspected urinary retention.	Large bladder was demonstrated.	Urine catheter was installed.
Four patients with clinical diagnosis of shock without appropriate response to intravenous fluids.	Hyperdynamic left ventricle and completely collapsible IVC.	Fluid management was adjusted according to changes IVC collapsibility and respiratory variations.

MVA-motor vehicle accident; LVEF-left ventricle ejection fraction; MR-mitral regurgitation; MS-mitral stenosis; CHF-congestive heart failure; PKD-polycystic kidney disease; LP-lumbar puncture; US-ultrasound; IVC- inferior vena cava.

suspected urine retention.

Respiratory variations of inferior vena cava diameter were used in four patients to assist clinical estimation of volume status (Fig. 5). Fluid management was adjusted according to changes in inferior vena cava (IVC) collapsibility and respiratory variations.

Two trauma patients were confirmed with hemorrhagic shock as a result of a hyperdynamic left ventricle and completely collapsible IVC; both were treated with fluids and blood, and promptly transferred.

Another patient presented with borderline blood pressure, fever, and large pericardial effusion. A collapsible IVC and absence of free wall diastolic collapse allowed the clinicians to differentiate sepsis from cardiac tamponade. The patient was treated with antibiotics and IV

fluids, with improvement of vital signs.

4. Discussion

The augmentation of physical examination using pocket-size US machine in rural Uganda led to significant clinical findings in 70% of cases and to change in case management in 87% of the cases. In 13% of the scanned patients, life-threatening findings were found (two positive FAST studies and one large pericardial effusion).

The need to develop simple, low-cost diagnostics for resource-limited settings are well recognized [16]. The fundamental characteristics of such technologies should include simplicity of operation, availability

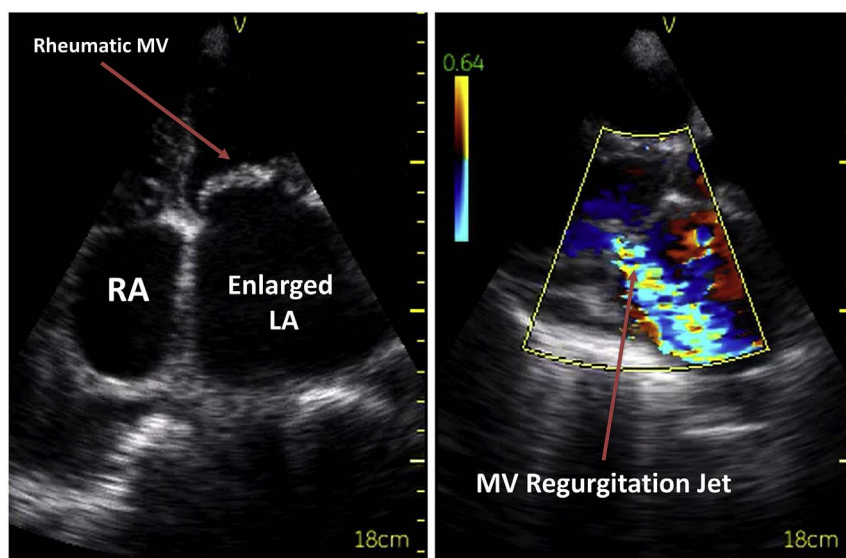


Fig. 2. Focused cardiac ultrasound performed on 40 years female admitted due to dyspnea and palpitations. Severe left ventricle dysfunction, left atrial dilatation and mitral valve regurgitation and stenosis, most probably of rheumatic origin, were diagnosed. RA-Right Atrium; LA- Left Atrium; MV- Mitral Valve.

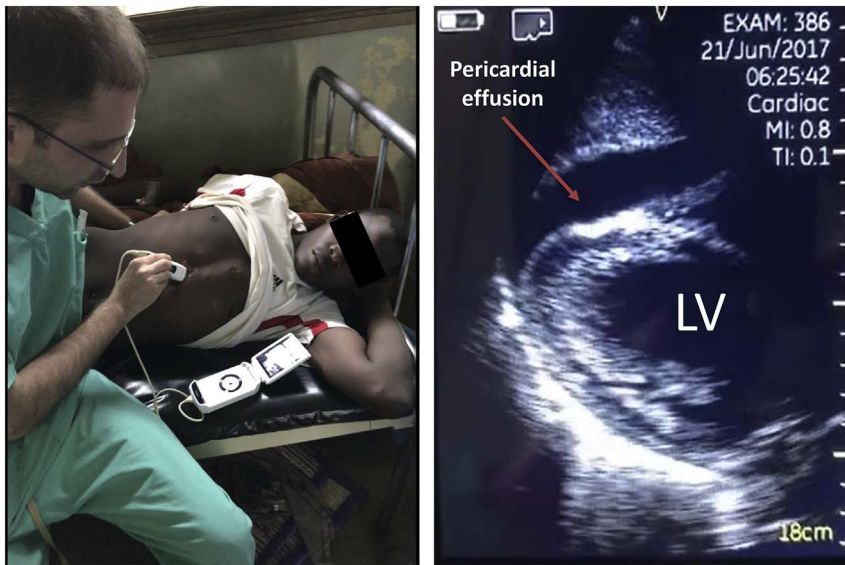


Fig. 3. Focused cardiac ultrasound performed on 24 years old male with fever, cough and chest pain. The study shows large pericardial effusion without signs of tamponade. LV- Left Ventricle.

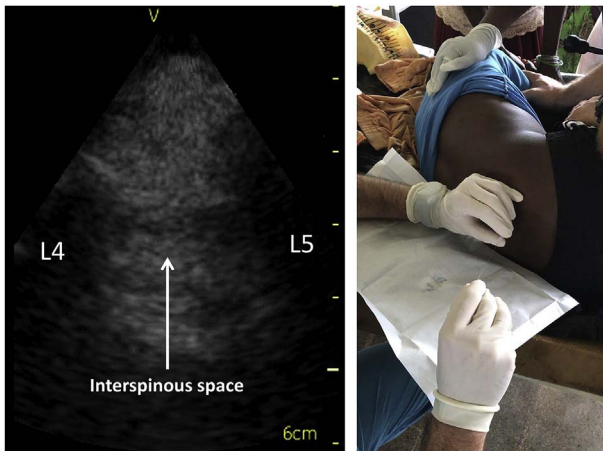


Fig. 4. Ultrasound guided lumbar puncture performed on 14 years old boy with suspected meningitis.

to primary-care physicians with basic training, relatively low cost, a steep learning curve, operability in areas with no consistent technical support, and a proven applicability to a wide variety of medical conditions. POCUS holds all of these qualities [5].

POCUS helps the physician to significantly augment the physical examination and identify abnormalities that could not be revealed without such technology [5]. These real-time dynamic images allow the findings to be directly correlated with the patient's presenting signs and symptoms, and enable a rapid change in management and placement of patients, especially in resource-limited rural medical centers.

A small preliminary study performed in a rural medical clinic staffed by local nurses in Nicaragua showed promising results of telemedicine-based ultrasound implemented into everyday practice [17]. Although the most common indications for scanning were pregnancy exclusion and abdominal pain, the authors describe change in management in 48% of the cases as a result of ultrasound examination. Few other studies, all performed in a simulated environment, have shown the ability of non-physicians, after a short training, to acquire high-quality ultrasound images and transfer them using various telemedicine methods [9,18,19].

This is the first description of the use of POCUS for acutely ill patients in rural medical centers with limited resources in sub-Saharan Africa, assisted by real-time long-distance professional review.

We believe that the main applications of POCUS, combined with telemedicine, include the following:

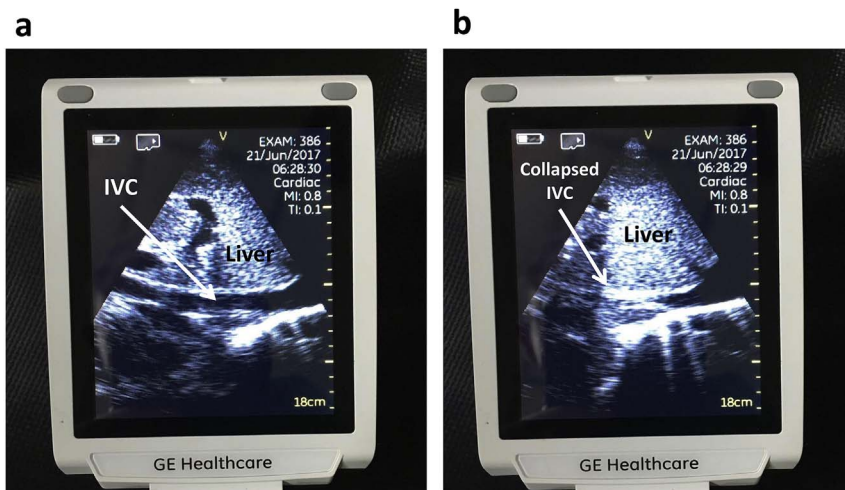


Fig. 5. Respiratory variations of inferior vena cava (IVC) diameter assessment performed on trauma patient with borderline blood pressure. The study demonstrates complete collapse of IVC with inspiration (b).

4.1. Trauma

In Africa, trauma is the third commonest cause of death.

The treatment of trauma casualties is complicated by limited diagnostic facilities combined with poor infrastructures. These factors make the referral of trauma patients to tertiary medical centers extremely time- and resource-consuming. Practically, only patients with a clear indication for urgent surgical intervention are transferred.

Hemorrhagic shock is identified promptly using POCUS. The triad of small, highly collapsible IVC, small hyperdynamic LV and RV in context of trauma is strongly suggestive of hemorrhagic shock. A FAST examination, demonstrating free intra-peritoneal fluid (Fig. 1), may be completed in less than 5 min and has been shown to have an overall accuracy of 90–98% for significant injury [5].

In patients with thoracic trauma, extension of the scan to subcostal view and chest enables rapid and accurate diagnosis of pericardial effusion and pneumothorax respectively [5].

The study can be repeated with any clinical deterioration and can either enhance patient transfer, shorten time of transfer to the operating room or help rule out significant internal bleeding [5].

4.2. Focused cardiac ultrasound

Heart failure is a major form of cardiovascular disease in sub-Saharan Africa [20]. While ischemic heart disease is quite uncommon, hypertension, rheumatic heart disease, infectious heart disease (mainly secondary to tuberculosis and HIV), endomyocardial fibrosis and cardiomyopathies contribute significantly to the development of heart failure in young adults in these regions [20,21].

POCUS can significantly improve cardiac physical examination [5]. Reduced left and right ventricle function, major valvulopathies, signs of volume overload (as reflected by enlarged and non-collapsible IVC), pericardial effusion and pleural effusion, as well as signs of lung congestion are identified by POCUS after relatively short training and by non-cardiologist personnel [22,23].

Figs. 2 and 3 present some of the cardiac findings that a resident was able to demonstrate in rural Uganda, where no other imaging modalities are available, after a basic five-day POCUS course.

The use of remote expert support via smartphone for echo image transfer has been shown to be feasible, with substantial reduction in the need for complete echo studies [24].

4.3. Lung ultrasound

Pneumonia is still the biggest killer in Africa [25]. The lack of prompt and accessible diagnostic tools and treatment for vulnerable populations, especially young children, adds to this burden [26].

Pleural effusion and empyema are common findings in Africa, and complicate as many as 15% of all pneumonia cases [27].

POCUS can readily replace plain chest x-ray in the majority of patients. US seems to be both more accurate and much cheaper than plain X-rays for identifying common lung pathologies [3]. Thoracic ultrasonography has a diagnostic accuracy similar to that of computed tomography in the imaging of pleural effusion (Fig. 6a), consolidation (Fig. 6b) and pneumothorax [5].

B-lines are a major characteristic of alveolar interstitial disease (Fig. 6c). Diffuse, bilateral B-lines can be caused by cardiogenic or noncardiogenic pulmonary edema, such as acute respiratory distress syndrome. In contrast, B-lines that are limited to one part of the lung are associated with lobar pneumonia, pulmonary contusion, or atelectasis [5].

POCUS has the potential ability to identify, characterize, quantify, and guide the drainage of pleural effusion [5]. Identification of complex, septated, or homogeneously echogenic fluid is highly correlated with empyema or hemorrhage and can guide decision making on whether to perform simple pleurocentesis or insert a chest drain

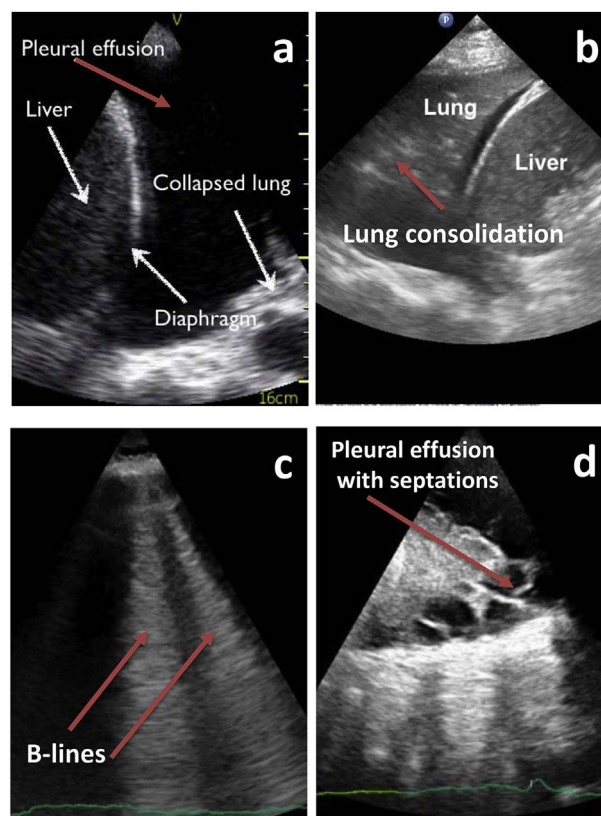


Fig. 6. Lung Ultrasound. a-pleural effusion; b-lung consolidation; c- B-lines; d-complex pleural effusion with septations suggestive for empyema.

(Fig. 6d) [28].

4.4. Differential diagnosis of shock

The great majority of hospitals in sub-Saharan Africa have no intensive-care facilities and the majority of severely sick or injured patients are treated in general medical wards with limited facilities [29]. However, sepsis, septic shock and hypovolemia are common, and many patients require prompt fluid resuscitation at admission.

POCUS is used for the real time assessment of shock [30]. Hypovolemic shock is relatively easily and rapidly identified as described above.

Cardiogenic shock is diagnosed by cardiac ultrasound at the bedside. The demonstration of poor LV function and enlarged non-collapsible IVC (reflecting elevated CVP due to LV failure) and lung B-lines (confirming pulmonary congestion) contribute to the diagnosis in the right clinical context [30].

Distributive shock, such as septic shock, is also demonstrated by POCUS. Hyperdynamic function of the LV and RV reflect the low systemic vascular resistance that is associated with distributive shock. In this context the POCUS can further identify the source of infection: lung consolidation, inflamed gallbladder, liver or spleen abscess, or pyelonephritis [17].

Obstructive shock is also readily identified by POCUS. Tamponade is suspected when there is consistent clinical presentation with effusion in the pericardial space. Collapsible free RA/RV wall during chamber diastole confirms the diagnosis of tamponade, and a non-collapsible large IVC confirms elevated right heart pressure (high CVP) [30]. The extended FAST, looking for pneumothorax, as described above, can confirm this reason for obstructive shock.

US can become an effective, bedside monitor after the first assessment of shock, and after an etiology is confirmed. Combination of repeat IVC assessment, end diastolic area of the LV and RV and lung US

can serve as a reliable method for guiding fluid administration, and enable the treating physician to avoid under resuscitation or fluid overload. Universal, unguided, fluid bolus administration to children with severe sepsis in Africa was associated with increased mortality in FEAST study [31].

4.5. Procedural guidelines

In the developing world, many bedside procedures are performed by physicians with limited experience, and sometimes with improvised equipment. The availability of tools for early detection of procedure success or complications (e.g. pneumothorax secondary to thoracentesis) is also limited.

POCUS has been shown to improve success and decrease complications in wide variety of procedures, including central and peripheral vascular access, thoracentesis, paracentesis, arthrocentesis, regional anesthesia, incision and drainage of abscesses, localization and removal of foreign bodies and lumbar puncture [5]. For example, using US to guide peripheral vascular access has provided a success rate between 91% and 97% after previous failed attempts; this technique is learned easily.

5. Conclusions

In conclusion, the presented cases demonstrate that, in a very resource-limited environment, POCUS conducted by basically trained primary care physicians, is a powerful diagnostic tool in variety of medical conditions. Its combination with the backup of experts using telemedicine seems to improve patient management and outcome, especially in resource-limited environments with a paucity of imaging modalities.

Although there is no consensus on the duration and extent of training required, we believe that there is a sufficient amount of evidence to suggest that investment in training programs directed to local health care providers in low-income countries, can have a positive impact on patient care through the use of specific scanning protocols and sufficient telemedicine support.

We encourage policy and decision makers to invest more in the advancement of radiology in low-income regions. In the context of POCUS, this should include increasing the availability of POCUS courses and machines, enabling telemedicine backup, and initiate larger cohort studies to evaluate the impact of this technology on patient outcome.

Authors' contribution to the study

DE, NP, EK, EM, EA, RH, ZSA, AM and LF had full access to all of the data in the study and take responsibility for the content of the manuscript, were involved in the conception, hypotheses delineation, and design of the study, acquisition and analysis of the data, in writing the article and in its revision prior to submission.

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Conflicts of interest

All the authors declare they have no conflict of interest related to this manuscript.

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