

Point-of-Care Ultrasound in the Diagnosis of Melioidosis in Laos

Michaëla A. M. Huson,^{1*†} Kerstin Kling,^{2,3,4†} Somaphone Chankongsin,⁵ Khampheng Phongluxa,⁶ Valy Keoluangkhot,⁵ Paul N. Newton,^{7,8,9} David Dance,^{7,8,9} Tom Heller,^{10‡} and Andreas Neumayr^{2,3‡}

¹Department of Microbiology and Infectious Diseases, Erasmus Medical Centre, Rotterdam, The Netherlands; ²Department of Medicine, Swiss Tropical and Public Health Institute, Basel, Switzerland; ³University of Basel, Basel, Switzerland; ⁴Department of Infectious Disease Epidemiology, Robert Koch-Institute, Berlin, Germany; ⁵Infectious Diseases Ward, Mahosot Hospital, Vientiane, Lao People's Democratic Republic; ⁶Lao Tropical and Public Health Institute, Ministry of Health, Vientiane, Lao People's Democratic Republic; ⁷Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU), Microbiology Laboratory, Mahosot Hospital, Vientiane, Lao People's Democratic Republic; ⁸Centre for Tropical Medicine and Global Health, University of Oxford, Oxford, United Kingdom; ⁹Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom; ¹⁰Lighthouse Clinics, Lilongwe, Malawi

Abstract. Melioidosis is endemic in many rural areas in Southeast Asia where facilities for culture and identification of *Burkholderia pseudomallei* are often limited. We performed a prospective observational study in patients presenting with fever to Mahosot Hospital, the primary referral hospital in Laos, to establish whether the detection of abscesses on ultrasound could support a presumptive diagnosis of melioidosis. All patients underwent ultrasound examination to detect abscesses in the liver, spleen, prostate, or, if indicated, subcutaneous tissue. We enrolled 153 patients, including 18 patients with melioidosis. Of these, 11 (61%) had an abscess at one or more sites, including five (28%) with splenic and/or liver abscesses. Absence of abscesses cannot rule out melioidosis, but the positive predictive value of abscesses for melioidosis was high at 93% (88–96%). Therefore, in endemic areas, the presence of abscesses in febrile patients should prompt empiric antibiotic therapy for melioidosis even in the absence of culture confirmation.

Over the past two decades, ultrasound has evolved as a noninvasive, reproducible, low-cost diagnostic method in many disciplines of clinical medicine. In addition, the development of low-priced transportable ultrasound machines has created opportunities in resource-constrained settings where other diagnostic imaging methods remain largely unavailable. Growing availability of portable ultrasound machines has also led to the establishment and implementation of numerous “point-of-care ultrasound” (POCUS) protocols in various medical disciplines, including tropical medicine.^{1–3} For example, in settings with a high prevalence of HIV and tuberculosis, the Focused Assessment with Sonography for HIV/TB (FASH) protocol is widely used to assess for signs of extrapulmonary tuberculosis.^{1,4} Melioidosis is an infectious disease caused by the Gram-negative bacterium *Burkholderia pseudomallei*. The disease is primarily reported in Southeast Asia and Northern Australia, but increasingly recognized to also occur in sub-Saharan Africa and South America.⁵ In parts of Southeast Asia, including Laos,⁶ melioidosis is an important differential diagnosis in patients with fever and/or sepsis. Risk factors for infection include diabetes, excessive alcohol use, chronic renal disease, chronic lung disease, thalassemia, malignancy, and other non-HIV-related immune suppression. Because the clinical presentation of melioidosis is often unspecific and the laboratory capacity for microbiological confirmation is frequently limited in endemic areas, establishing the diagnosis remains challenging, and many cases are likely missed. However, because abscess formation (especially in the liver, spleen, and soft tissue, although prostatic abscesses were additionally reported in Northern Australia) is frequently observed in melioidosis and easily assessable by ultrasound,^{7–9} we hypothesized that POCUS might provide a valuable adjunct diagnostic tool to corroborate the diagnosis

and allow early initiation of effective antimicrobial treatment. The latter is critical because melioidosis has an overall case fatality rate of up to 40%,⁹ and *B. pseudomallei* requires treatment with ceftazidime or a carbapenem, two drugs not often used as first-line empiric treatment of sepsis.¹⁰

This study on melioidosis was part of a larger observational diagnostic study, investigating the value of POCUS in the clinical management of febrile patients admitted to Mahosot Hospital, the primary reference hospital of Laos, in Vientiane. The study was prospectively performed in the hospital's adult infectious diseases ward, enrolling consecutively admitted febrile patients between August 2016 and December 2016. Fever on admission was the sole inclusion criterion, and inability to undergo an ultrasound examination and unwillingness to participate in the study were the only exclusion criteria.

Patient data were collected on detailed case report forms and entered into an electronic database; the data were cleaned by checking for consistency and missing data. The POCUS examinations were performed using a portable black-and-white ultrasound machine (DP-30 with 3.5 MHz-convex and 7.5 MHz linear probe, Mindray, China) by a study clinician (K. K.) as soon as possible after hospital admission, usually on the day of admission. A description of the standard operating procedure for the POCUS examination is provided in Supplemental Appendix 1. In brief, an ultrasound examination assessing for pericardial and pleural effusions, ascites, abdominal lymphadenopathy, and abscesses in solid organs was performed using a convex probe. A linear probe was additionally used to assess the spleen for small abscesses and evaluate soft tissue abscesses when present. Sonographic findings were saved as digital pictures in patients with normal findings and as video clips in patients with abnormal findings. The Mahosot Hospital radiology department provided timely on-site expertise and served as a reference in the event of unclear sonographic findings. In addition, video clips of abnormal findings were reviewed externally (T. H.) to ensure quality control. Data were analyzed using descriptive statistics. For data analysis, we only used POCUS results of

* Address correspondence to Michaëla A. M. Huson, Department of Microbiology and Infectious Diseases, Erasmus Medical Centre, Rotterdam, The Netherlands. E-mail: m.huson@erasmusmc.nl

† These authors contributed equally to this work.

‡ These authors contributed equally to this work.

patients with a definite diagnosis, obtained using data from routine clinical care according to the locally available and established diagnostic standards, comparing patients with melioidosis and those with an alternative diagnosis. Confirmed melioidosis was defined by a positive *B. pseudomallei* culture result from any clinical sample (e.g., blood, urine, throat swab, sputum, and pus) conducted at the microbiology laboratory of Mahosot Hospital. *P*-values were calculated using the Mann–Whitney U-test for continuous variables and Fisher's exact *t*-test for categorical variables. The study was approved by the National Ethics Committee for Health Research in Laos and by the Ethics Committee of Northwest and Central Switzerland. Written informed consent was obtained from all study participants or their legal guardians.

During the study period, 153 patients were included of whom 76 were men. In 111 patients, a definite diagnosis was obtained, including microbiologically confirmed melioidosis in 18 patients. Clinical characteristics and ultrasound findings are shown in Table 1. Patients with melioidosis were older than patients with an alternative diagnosis and were more likely to have underlying diabetes mellitus and less likely to be HIV positive. Pathological findings on ultrasound were generally common in patients both with and without melioidosis. However, the presence of abscesses was significantly more common in patients with melioidosis (Table 1, Figure 1). Eleven (61%) patients with melioidosis had an abscess at one or more sites compared with one (1%) patient with an alternative diagnosis ($P < 0.0001$). The positive and negative predictive value of finding one or more abscesses in patients with melioidosis in our patient population was 92% (60–99%) and 93% (88–96%), respectively.

Our findings provide information on the diagnostic accuracy of positive ultrasound findings (abscesses) to diagnose

melioidosis in patients admitted with fever in Laos and are in line with previous studies on ultrasound findings in patients with melioidosis in Southeast Asia. A retrospective study in patients with culture-proven melioidosis in India demonstrated that 23/189 (12%) had liver abscesses, a similar proportion to the 11% in our study.¹¹ In Thailand, a prospective observational study in 230 patients with culture-confirmed melioidosis found one or more abscesses in the liver and/or spleen in 77 (33%) patients,¹² similar to the 28% observed in our study. Other studies have demonstrated that the combination of liver and splenic abscesses can be predictive for melioidosis.^{7,13} In our study, the number of patients with multiple abscesses was low, but three of four patients with multiple abscesses were confirmed with melioidosis. In two patients with prostatic abscesses and three patients with splenic abscesses, including one patient who also had liver abscesses, no definite diagnosis was made. Possibly, these patients were true cases of melioidosis where a firm diagnosis might have been reached had it been possible to aspirate the abscesses. We found only one patient with a hypoechoic liver lesion who had a confirmed alternative diagnosis. This patient was found to have dengue fever. As dengue fever does not cause abscesses, another infection or a noninfectious pathology may have been present.

Four main limitations of our study need to be mentioned. First, the sample size was limited. Second, the diagnostic accuracy of ultrasound was calculated based on the analysis of culture confirmed cases of melioidosis. Because the sensitivity of culture is not perfect and some referred patients may have received antibiotic treatment before diagnostic blood sampling, some cases may have been missed. Third, our study was carried out during the dengue season. This may have increased our positive predictive value as a large

TABLE 1
Point-of-care ultrasound findings in patients admitted with fever in Vientiane, Laos

	All patients (n = 153)	Patients with melioidosis (n = 18)	Patients with alternative diagnosis* (n = 93)	P-value†
Baseline characteristics				
Men (%)	76 (50)	11 (61)	42 (45)	0.14
Median age (years) (IQR)	30 (22–42)	53 (45–62)	29 (22–38)	< 0.0001
Diabetes	16 (10)	8 (44)	4 (4)	< 0.0001
HIV positive (%)	36 (24)	0 (0)	23 (25)	0.02
Ultrasound findings (%)				
Positive ultrasound findings	80 (52)	10 (56)	45 (48)	0.62
Pericardial effusion‡	20 (14)	1 (6)	11 (12)	0.69
Abdominal lymph nodes	6 (4)	0 (0)	4 (4)	1.0
Pleural effusion	50 (33)	5 (28)	23 (25)	0.77
Intra-abdominal effusion	25 (16)	0 (0)	9 (10)	0.35
Gall bladder edema	10 (7)	1 (6)	7 (8)	1.0
Liver abscess§	4 (3)	2 (11)	1 (1)	0.07
Spleen abscess§	7 (5)	4 (22)	0 (0)	0.0005
Prostate abscess§	3 (2)	1 (6)	0 (0)	0.16
Soft tissue abscess§	8 (5)	7 (39)	0 (0)	< 0.0001
Composite ultrasound findings (%)				
Any abscess¶	18 (12)	11 (61)	1 (1);	< 0.0001
Abscesses in multiple sites¶#	4 (3)	3 (17)	0 (0)	0.004
Liver and/or splenic abscess	9 (6)	5 (28)	1 (1);	0.0004

* Most common alternative diagnoses included dengue (n = 47), rickettsiosis (n = 13), HIV-related opportunistic disease (n = 11), and urinary tract infection (n = 6).

† All *P*-values are provided for the comparison between patients with melioidosis and those with an alternative diagnosis. Some patients did not have a definite final diagnosis; they were not included in the analysis.

‡ All pericardial effusions observed in this study were relatively small, with a maximum diameter of 12 mm.

§ Hypoechoic lesions on ultrasound were interpreted as abscesses.

|| This patient had a confirmed diagnosis of dengue fever. This diagnosis does not explain the presence of a liver abscess, but unfortunately invasive microbiological diagnostics to establish the cause of the liver abscess were unavailable.

¶ Including liver, splenic, prostatic, and soft tissue abscesses.

Abscesses in multiple sites were only observed in patients with melioidosis and in one patient without a definitive diagnosis. In this patient, ultrasound demonstrated liver and splenic abscesses, suggesting melioidosis despite negative culture results.

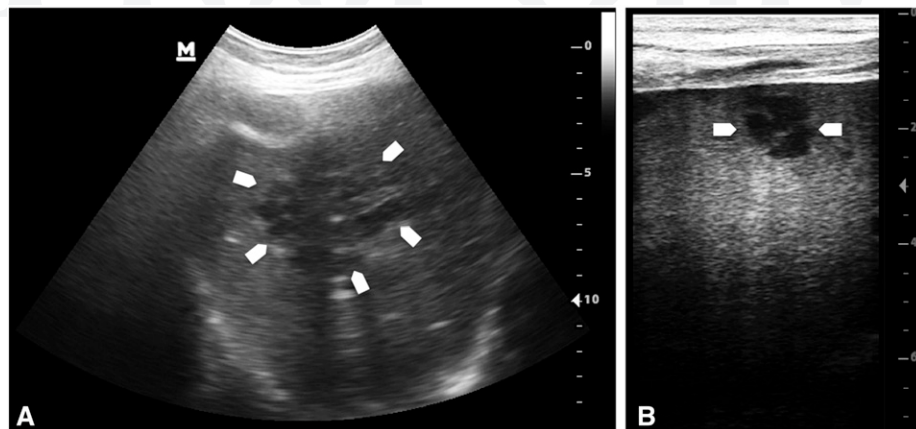


FIGURE 1. Hypoechoic lesions (arrows) due to melioidosis abscesses in the (A) liver (3.5 MHz convex) and (B) spleen (7.5 MHz linear probe). The hypoechoic lesions in the liver and spleen were frequently septated and/or localized in clusters which may suggest “honeycomb” or “necklace” appearance; because of the low image quality of the point-of-care ultrasound machine, these possibly diagnostic characteristics of the ultrasound images were not included in the analysis.

proportion of our control group were patients with dengue infection. In other seasons, the proportion of febrile patients with alternative causes of abscesses such as amebic disease or staphylococcal infection may be higher. Finally, we used a low-cost ultrasound machine, which may have limited the detection of very small abscesses as well as the morphological characteristics suggestive of melioidosis (such as the “necklace” or “honeycomb” appearance previously described in melioidosis).¹¹ Nevertheless, we intentionally opted for using simple equipment and simple image characteristics, as these more realistically reflect the conditions under which POCUS is applied. Furthermore, by adding a linear probe for screening of the spleen and soft tissue, we allowed for more sensitive detection of small abscess. However, because of the small sample size, it is difficult to ascertain whether morphological features are helpful in strengthening the tentative diagnosis of melioidosis in settings comparable with ours.

Although ultrasound cannot be used to rule out melioidosis, as not all patients with melioidosis develop abscesses, the presence of abscesses had a high positive predictive value in our study population. This cannot necessarily be extrapolated to other places and time periods, depending on the local epidemiology of other conditions such as amebic liver disease and staphylococcal infection. An additional differential diagnosis includes abscesses due to disseminated tuberculosis, although the patient populations at risk are significantly different. Whereas underlying diabetes and renal impairment may suggest melioidosis, HIV infection and malnutrition may indicate tuberculosis. Nevertheless, these differentials illustrate the limitations of imaging and the need for microbiological confirmation in equivocal cases. However, our data indicate that in a melioidosis-endemic area, the detection of abscesses in the liver, spleen, and soft tissue by bedside ultrasound has a high positive predictive value for melioidosis, especially when abscesses are detected in multiple sites in high-risk patients. In these patients, empiric antibiotic treatment covering *B. pseudomallei* should, thus, be initiated even in the absence of positive cultures.

Received January 27, 2020. Accepted for publication March 16, 2020.

Note: Supplemental appendix appears at www.ajtmh.org.

Acknowledgment: We thank all patients who participated in this research for their willingness to contribute.

Financial support: This study was entirely financed by the authors and did not receive external funding.

Authors' addresses: Michaëla A. M. Huson, Department of Microbiology and Infectious Diseases, Erasmus Medical Centre, Rotterdam, The Netherlands, E-mail: m.huson@erasmusmc.nl. Michaëla Huson, Department of Infectious Disease Epidemiology, Robert Koch-Institute, Berlin, Germany, E-mail: klngk@rki.de. Somaphone Chankongsin and Valy Keoluangkhot, Infectious Diseases Ward, Mahosot Hospital, Vientiane, Lao People's Democratic Republic, E-mails: sona_der@yahoo.com and valy.keoluangkhot@gmail.com. Khampheng Phongluxa, Lao Tropical and Public Health Institute, Ministry of Health, Vientiane, Lao People's Democratic Republic, E-mail: khampheng_p@hotmail.com. Paul N. Newton and David Dance, Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU), Microbiology Laboratory, Mahosot Hospital, Vientiane, Lao People's Democratic Republic, E-mails: paul.newton@tropmedres.ac and david.d@tropmedres.ac. Tom Heller, Lighthouse Clinics, Lilongwe, Malawi, E-mail: echnatom@web.de. Andreas Neumayr, Department of Medicine, Swiss Tropical and Public Health Institute, Basel, Switzerland, E-mail: andreas.neumayr@swisstph.ch.

REFERENCES

- Heller T, Wallrauch C, Goblirsch S, Brunetti E, 2012. Focused assessment with sonography for HIV-associated tuberculosis (FASH): a short protocol and a pictorial review. *Crit Ultrasound J* 4: 21.
- Bélar S, Tamarozzi F, Bustinduy AL, Wallrauch C, Grobusch MP, Kuhn W, Brunetti E, Joekes E, Heller T, 2016. Point-of-care ultrasound assessment of tropical infectious diseases—a review of applications and perspectives. *Am J Trop Med Hyg* 94: 8–21.
- Henwood PC, Mackenzie DC, Liteplo AS, Rempell JS, Murray AF, Leo MM, Dukundane D, Dean AJ, Rulisa S, Noble VE, 2017. Point-of-care ultrasound use, accuracy, and impact on clinical decision making in Rwanda hospitals. *J Ultrasound Med* 36: 1189–1194.
- van Hoving DJ, Lamprecht HH, Stander M, Vallabh K, Fredericks D, Louw P, Müller M, Malan JJ, 2013. Adequacy of the emergency point-of-care ultrasound core curriculum for the local burden of disease in South Africa. *Emerg Med J* 30: 312–315.
- Birmie E, Virk HS, Savelkoel J, Spijker R, Bertherat E, Dance DAB, Limmathurotsakul D, Devleeschauwer B, Haagsma JA, Wiersinga WJ, 2019. Global burden of melioidosis in 2015: a systematic review and data synthesis. *Lancet Infect Dis* 19: 892–902.

6. Dance DAB, Luangraj M, Rattanavong S, Sithivong N, Vongnalaysane O, Vongsouvath M, Newton PN, 2018. Melioidosis in the Lao People's Democratic Republic. *Trop Med Infect Dis* 3: ■.
7. Muttarak M, Peh WC, Euathrongchit J, Lin SE, Tan AG, Lerttumnongtum P, Sivasomboon C, 2009. Spectrum of imaging findings in melioidosis. *Br J Radiol* 82: 514–521.
8. Alsaif HS, Venkatesh SK, 2016. Melioidosis: spectrum of radiological manifestations. *Saudi J Med Med Sci* 4: 74–78.
9. White NJ, 2003. Melioidosis. *Lancet* 361: 1715–1722.
10. Dance D, 2014. Treatment and prophylaxis of melioidosis. *Int J Antimicrob Agents* 43: 310–318.
11. Kiangte HL, Vimala LR, Eapen A, Veeraraghavan B, Karuppusami R, Gibikote S, 2018. A retrospective case-control study to evaluate the diagnostic accuracy of honeycomb sign in melioid liver abscess. *Am J Trop Med Hyg* 99: 852–857.
12. Maude RR et al., 2012. Prospective observational study of the frequency and features of intra-abdominal abscesses in patients with melioidosis in northeast Thailand. *Trans R Soc Trop Med Hyg* 106: 629–631.
13. Apisarnthanarak A, Apisarnthanarak P, Mundy LM, 2006. Computed tomography characteristics of *Burkholderia pseudomallei* liver abscess. *Clin Infect Dis* 42: 989–993.

Supplemental Appendix 1: Standard operating procedure for Point-of-care ultrasound (POCUS)

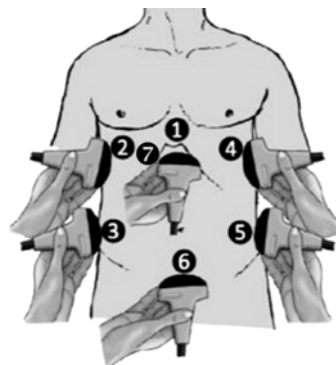
1. Equipment:

- Bed: For the ultrasound examination the patient has to be in a supine position and the examiner needs to be on the patient's right side.
- Ultrasound machine model: a portable Mindray DP-20 will be used for all ultrasound investigations.
- Probes: Besides the probe position assessing the spleen, an electronic convex array transducer (35C50EB) will be used in all probe positions; in the probe position assessing the spleen an electronic linear array transducer (75L53EA) will be used. If soft-tissue abscesses are present the linear probe will be used to assess them.
- Ultrasound gel: Ultrasound gel is used as conductive medium between the patient's skin and the ultrasound transducer and will be used in every ultrasound examination.

2. Procedural Steps

- All eligible patients fulfilling the study's inclusion criteria will be invited to participate in the study.
- Eligible patients willing to participate will be enrolled after they have been informed on the study's aim and after the ultrasound procedure has been explained.
- The lights in the room will be turned off and the room darkened, if possible.
- The patient will be placed in a supine position and the ultrasound machine set up on the right side of the patient.
- The patient's study number will be registered in the ultrasound machine prior to starting the examination.
- A reasonable amount of ultrasound gel will be put on the probe.
- The bedside ultrasound examination will be started following the protocol specified below.

3. Scanning positions



Position 1A: pericardial effusion

- Probe position:* The probe is placed transverse in the epigastric angle and the transducer is then tilted cranially to obtain a view on the heart. Asking the patient to inspire help to displace the heart caudally and improve visualization.
- Normal finding:* The pericardium is seen as an echogenic structure above the liver and diaphragm and below the heart; the parietal and visceral pericardium are inseparable.

- c. *Pathological finding:* Pericardial effusion shows as an anechoic, black rim around the heart separating the parietal and visceral pericardium. Within the pericardial fluid echogenic material from inflammatory stranding may be seen. Isolated fluid at the apex is a normal finding and will not be reported as pericardial effusion.
- d. *Interpretation:* An anechoic rim surrounding the heart confirms pericardial effusion and will be documented as such.

Position 1B: abdominal lymphnodes

- a. *Probe position:* Four standard planes will be applied to visualize abdominal lymphnodes: 1) oblique porta hepatis, 2) transverse through head of pancreas, 3) splenic hilar, and 4) transverse mid abdomen.
- b. *Normal finding:* Many physiological structures going beyond the scope of FASH can be seen.
- c. *Pathological finding:* Lymphnodes appear as hypoechoic round or oval structures increasing in size as the transducer moves towards the centre of the node, and then diminishing again as the opposite side is reached. The nodes will be measured in their short axis. Central necrosis of lymphnodes may be seen.
- d. *Interpretation:* In children, lymph nodes >10 mm (short axis) will be considered pathological and documented respectively. In adults, lymph nodes >15 mm (short axis) will be considered pathological and documented respectively. If present, central necrosis of lymphnodes will be documented.

Position 2: pleural effusion right side

- a. *Probe position:* After the patient is asked to put his arms behind the neck, the transducer is positioned dorsal of the right mid-axillary line at the caudal part of the thorax with the long axis parallel to the ribs.
- b. *Normal findings:* The diaphragm and the apical parts of the liver will be seen. Air in the basal parts of the lung will cause artefacts resembling a curtain that moves up and down with respiration.
- c. *Pathological findings:* Anechoic black fluid may be seen in the costo-phrenic angle; within the pleural fluid echogenic material from inflammatory stranding may be seen. Hepatized lung tissue, the absence of A-lines and bronchograms may be seen.
- d. *Interpretation:* Fluid in the costo-phrenic angle will be documented as pleural effusion. Hepatized lung tissue with absence of A-lines and possible concurrent bronchograms will be documented as lung consolidation.

Position 3A: ascites in the hepato-renal pouch (Morrison's Pouch)

- a. *Probe position:* The transducer is moved a few centimetres caudally and rotated so that the longitudinal axis is parallel to the body long axis.
- b. *Normal finding:* The caudal edge of the liver and the kidney are visible with an echogenic white line between them.
- c. *Pathological finding:* Anechoic fluid is visible between the liver and the kidney or around the kidney. Echogenic material from inflammatory processes may be floating within the fluid.
- d. *Interpretation:* Presence of free fluid will be documented as ascites.

Position 3B: right kidney

- a. *Probe position:* The probe is placed in the right lower intercostal space in the midaxillary line and the liver may be used as "acoustic window". After assessing the kidney in the longitudinal

view (long axis) the probe is rotated and the kidney is scanned in the transverse (short axis) view to visualize the whole organ.

- b. *Normal finding:* The kidney is invested in an echogenic capsule (Gerota's fascia) surrounded by perinephritic fat, which in turn is surrounded by fascia. The kidney may be divided in renal cortex and medulla (parenchyma with a similar texture as liver tissue) which surrounds the echogenic renal sinus consisting of the pelvicalyceal system, renal vessels and fat. The calyces unite in the renal pelvis, which is the funnel-shaped origin of the ureter. The ureters are generally not well visualized, unless distended.
- c. *Pathological finding:* Renal calculi / stones are brightly echogenic and demonstrate posterior acoustic shadowing. Dilatation of the renal pelvis and calyces (Hydronephrosis) points to an obstructed outflow of urine.
- d. *Interpretation:* Presence of stones and hydronephrosis will be documented.

Positions 3C: focal liver lesions

Position 3C-I: liver longitudinal

- a. *Probe position:* After the patient is asked to put his arms behind the neck, the probe is placed longitudinal in the epigastric angle and the transducer is then tilted to the left assessing the left liver lobe. Then the transducer is sliding to the right (finally ending at the right flank) trying to visualize as much liver tissue as possible. Asking the patient to inspire (if age appropriate) may help to displace the liver caudally and improve visualization.
- b. *Normal finding:* The liver is seen as a moderately echogenic homogenous organ. Structures above the liver are the diaphragm and the heart; anechoic normal structures are intrahepatic vessels and the gallbladder.
- c. *Pathological finding:* Focal non-linear hypoechoic lesions within the liver tissue (e.g. tuberculous granuloma or abscesses).
- d. *Interpretation:* Focal non-linear hypoechoic lesions, possibly depicting tuberculous granuloma or abscesses, will be documented. In addition, the number, size, distribution and morphology of these lesions will be documented.

Position 3C-II: liver transcostal

- a. *Probe position:* The transducer is moved slightly up and turned counter-clockwise until parallel to the ribs (intercostal window) to visualize the liver tissue. Similar images as in the positions 3b-I are achieved.
- b. *Normal findings:* The liver is seen as a moderately echogenic homogenous organ and linear hypoechoic vessels are seen. The air above the pleura may be seen as echogenic artefact.
- c. *Pathological finding:* Focal non-linear hypoechoic lesions within the liver tissue (e.g. tuberculous granuloma or abscesses).
- d. *Interpretation:* Focal non-linear hypoechoic lesions, possibly depicting tuberculous granuloma or abscesses, will be documented. In addition, the number, size, distribution and morphology of these lesions will be documented.

Position 3C-III: liver subcostal

- a. *Probe position:* The probe is placed transverse in the epigastric angle and the transducer is then tilted cranially to caudally to obtain a view on the liver. Asking the patient to inspire (if age appropriate) may help to displace the liver caudally and improve visualization.

- b. *Normal finding:* The liver is seen as a moderately echogenic homogenous organ. Structures above the liver are the diaphragm and the heart; anechoic normal structures are intrahepatic vessels (hepatic veins and portal vein) and the gallbladder.
- c. *Pathological finding:* Focal non-linear hypoechoic lesions within the liver tissue (e.g. tuberculous granuloma or abscesses).
- d. *Interpretation:* Focal non-linear hypoechoic lesions, possibly depicting tuberculous granuloma or abscesses, will be documented. In addition, the number, size, distribution and morphology of these lesions will be documented.

Position 4: pleural effusion left side

- a. *Probe position:* Mirroring position 2 the transducer is placed on the left side of the caudal thorax dorsal of the right mid-axillary line with the long axis parallel to the ribs.
- b. *Normal findings:* The diaphragm and spleen will be seen and in the absence of pleural effusion air in the basal parts of the lung will cause artefacts resembling a curtain that moves up and down with respiration.
- c. *Pathological findings:* Anechoic black fluid may be seen in the costo-phrenic angle; within the pleural fluid echogenic material from inflammatory stranding may be seen. Hepatized lung tissue, the absence of A-lines and bronchograms may be seen.
- d. *Interpretation:* Fluid in the costo-phrenic angle will be documented as pleural effusion. Hepatized lung tissue with absence of A-lines and possible concurrent bronchograms will be documented as lung consolidation.

Position 5A: focal splenic lesions

- a. *Probe position:* The transducer is moved slightly upwards and paralleled to the ribs to visualize the spleen.
- b. *Normal findings:* Homogenous tissue of the spleen is visible.
- c. *Pathological findings:* Hypoechoic dark lesions are visible within the splenic tissue.
- d. *Interpretation:* Hypoechoic dark lesions, possibly depicting abscesses, will be documented.

Position 5B: ascites in the spleno-renal pouch (Koller's Pouch)

- a. *Probe position:* The transducer is moved caudally mirroring position 3a and visualizing spleen and left kidney.
- b. *Normal findings:* Spleen and kidney appear separated by a white echogenic line representing the capsules of the organs.
- c. *Pathological findings:* Analogous to the hepato-renal pouch free fluid will be seen as black anechoic fluid in the spleno-renal pouch.
- d. *Interpretation:* Free fluid in the spleno-renal pouch will be documented as ascites.

Position 5C: left kidney

- a. *Probe position:* The transducer positioned and moved mirroring position 3b.
- b. *Normal finding:* see 3b.
- c. *Pathological finding:* see 3b.
- d. *Interpretation:* see 3b.

Position 6A: ascites in the pouch of Douglas (female) or recto-vesical pouch (male)

- a. *Probe position:* The probe is placed on the lower abdomen touching the upper of the symphysis pubis. The pelvic region will be scanned in the longitudinal and transverse axis.

- b. *Normal findings:* The bladder will be visible and varying in size depending urine content. In female patients the uterus may be visible as a “pear shaped” organ behind the bladder. In male patients the recto-vesical pouch is located between the rectum and the bladder.
- c. *Pathological findings:* Echo-free black areas may be seen behind the bladder (male patients) or uterus (female patients) and represent free fluid in the recto-vesical and Douglas pouch, respectively.
- d. *Interpretation:* Free fluid in the recto-vesical or Douglas pouch represents ascites and will be documented as such.

Position 6B: focal prostatic lesions

- a. *Probe position:* The probe will be tilted to scan the prostate located caudally to the bladder.
- b. *Normal findings:* Homogenous tissue of the round- to oval-shaped prostate is visible.
- c. *Pathological findings:* Hypoechoic dark lesions visible within the prostatic tissue.
- d. *Interpretation:* Hypoechoic dark lesions, possibly depicting abscesses, will be documented.

Position 7: gall bladder

- a. *Probe position:* Identify the gall bladder by a longitudinal scan in the mid-axillary line at the costal margin (altering the probe angle to scan between the ribs may be helpful) or place the probe subcostal and sweep down and laterally.
- b. *Normal finding:* The gall bladder appears as well-demarcated fluid-filled structure inferior to the liver. Normal gall bladder wall thickness is ≤ 3 mm in patients who fasted.
- c. *Pathological finding:* Calculi / stones are brightly echogenic and demonstrate posterior acoustic shadowing. A thickened wall often appears as two echogenic lines with a hypoechoic region between them. This suggests acute inflammation and/or oedema. Besides cholecystitis, oedema is also seen in sepsis and capillary leakage due to dengue. CAVE: The gall bladder wall will also be thickened due to contraction in non-fasted patients!
- d. *Interpretation:* The presence of calculi / stones and the presence of a thickened gall bladder wall in patients who fasted will be documented.

Soft-tissue abscesses

In the case apparent soft-tissue abscess(es) are present their number, location and size will be assessed by ultrasound and recorded, respectively.

4. Documentation

4.1 Document of POCUS results

- The findings of the POCUS examination will be documented in the "Laos-POCUS-study - Sonographical CRF"
- The positive and negative findings will be documented for each probe position, respectively.
- In the case of parenchymal liver and spleen lesions, their number, distribution, size and morphology will be recorded. The distribution of lesions was classified as "discrete" for scattered lesions separated by intervening normal parenchyma and "clustered" for multiple lesions located close together.
- The lesions will be documented as "hypoechoic" for simple round, hypo- to anechoic lesions without internal structure, "target" for small, hypoechoic lesions with a tiny central echogenic spot, "bull's eye" for lesions with a larger central echogenic area, "satellites" for lesions with multiple smaller surrounding lesions, "honey-comb" for lesions with internal septa or locules that might represent coalescence of multiple small lesions to form a large abscess and

"necklace" for multiple peripheral radial loculations contained within the larger hypoechoic honeycomb lesions. Schematic drawings are given below. Comments are possible, including free-text descriptions of the lesions' morphology in the CRF.

- The size of the lesions will be documented by using the digital measuring tool of the ultrasound machine.



Figure 2. From left to right: "hypoechoic", "target", "bull's eye", "satellite", "honey-comb", "necklace" shape of focal lesions in the liver

4.2 Documentation of visualisation conditions

The general visualisation conditions during the POCUS examination will be documented categorizing visualisation into "satisfactory", "partly obscured" and "obscured".

4.3 Saving of ultrasound scans

In every position of the POCUS examination, a scan picture will be electronically saved on the ultrasound machine's internal harddisk to document normal as well as pathological findings. Pathological findings will additionally be saved as video clips on the ultrasound machine's internal harddisk.