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Lung ultrasound findings in hospitalized COVID-19 patients in relation to venous thromboembolic events: the ECHOVID-19 study

Kristoffer Grundtvig Skaarup[1] · Mats Christian Højbjerg Lassen[1] · Caroline Espersen[1] · Jannie Nørgaard Lind[1] · Niklas Dyrby Johansen[1] · Morten Sengeløv[1] · Alia Saed Alhakak[1] · Anne Bjerg Nielsen[1] · Kirstine Ravnkilde[1] · Raphael Hauser[1] · Liv Borum Schöps[1] · Eva Holt[1] · Henning Bundgaard[2] · Christian Hassager[2] · Reza Jabbari[2] · Jørn Carlsen[2] · Ole Kirk[3] · Uffe Bodtger[4] · Matias Greve Lindholm[5] · Lothar Wiese[6] · Ole Peter Kristiansen[7] · Emil Schwarz Walsted[8] · Olav Wendelboe Nielsen[7] · Birgitte Lindegaard[9] · Niels Tønder[10] · Klaus Nielsen Jeschke[11] · Charlotte Suppli Ulrik[11] · Morten Lamberts[1] · Pradeesh Sivapalan[12] · Jannik Pallisgaard[1] · Gunnar Gislason[1] · Kasper Iversen[1] · Jens Ulrik Stæhr Jensen[12] · Morten Schou[1] · Søren Helbo Skaarup[13] · Elke Platz[14] · Tor Biering-Sørensen[1,15]

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Abstract

Purpose Several studies have reported thromboembolic events to be common in severe COVID-19 cases. We sought to investigate the relationship between lung ultrasound (LUS) findings in hospitalized COVID-19 patients and the development of venous thromboembolic events (VTE).

Methods A total of 203 adults were included from a COVID-19 ward in this prospective multi-center study (mean age 68.6 years, 56.7% men). All patients underwent 8-zone LUS, and all ultrasound images were analyzed off-line blinded. Several LUS findings were investigated (total number of B-lines, B-line score, and LUS-scores).

Results Median time from admission to LUS examination was 4 days (IQR: 2, 8). The median number of B-lines was 12 (IQR: 8, 18), and 44 (21.7%) had a positive B-line score. During hospitalization, 17 patients developed VTE (4 deep-vein thrombosis, 15 pulmonary embolism), 12 following and 5 prior to LUS. In fully adjusted multivariable Cox models (excluding participants with VTE prior to LUS), all LUS parameters were significantly associated with VTE (total number of B-lines: HR = 1.14, 95% CI (1.03, 1.26) per 1 B-line increase), positive B-line score: HR = 9.79, 95% CI (1.87, 51.35), and LUS-score: HR = 1.51, 95% CI (1.10, 2.07), per 1-point increase). The B-line score and LUS-score remained significantly associated with VTE in sensitivity analyses.

Conclusion In hospitalized COVID-19 patients, pathological LUS findings were common, and the total number of B-lines, B-line score, and LUS-score were all associated with VTE. These findings indicate that the LUS examination may be useful in risk stratification and the clinical management of COVID-19. These findings should be considered hypothesis generating.

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Keywords COVID-19 · Lung ultrasound · B-lines · Venous thromboembolic events

Introduction

COVID-19 is associated with the development of acute respiratory distress syndrome (ARDS), intensive care unit admission, multi-organ failure, and death, especially in the elderly [1, 2]. Elevated D-dimer and dysregulated coagulation have been reported in a significant proportion of hospitalized COVID-19 patients in multiple studies [3, 4] and found to be associated with a more severe course of the disease [5, 6]. As the pandemic has progressed,

KristofferGrundtvig Skaarup and MatsChristianHøjbjerg Lassen contributed equally.

All the authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

✉ Tor Biering-Sørensen
tor.biering-soerensen@regionh.dk

Extended author information available on the last page of the article

multiple case-reports, reviews, and observational studies have reported an increased incidence of venous thromboembolic events (VTE) in hospitalized COVID-19 patients [7–12]. It has been proposed that the increased risk of VTE is caused by a diffuse pulmonary intravascular coagulopathy resulting from pulmonary macrophage activation syndrome. In addition, a degree of cytokine storm-induced disseminated intravascular coagulation may add to the diffuse pulmonary intravascular coagulopathy beyond that of the macrophage activation [13, 14]. Moreover, disease severity is associated with prolonged periods of inactivity which in turn increases the risk of VTE. Thus, it has been suggested that COVID-19 patients should be monitored for possible development of VTE [15].

Lung ultrasound (LUS) is a sensitive method for detecting pneumonia and ARDS [16, 17] and is an inexpensive, fast, and readily available technique that can be performed bedside. The COVID-19 induced increased pulmonary coagulopathy causes pulmonary extravasation of fluid which is detectable by both LUS as B-lines and consolidations and by computed tomography (CT) [11, 18, 19]. In addition, CT-studies have shown that most pulmonary lesions related to COVID-19 are distributed peripherally in the lungs causing pulmonary edema detectable with LUS [20, 21]. Initial reports have demonstrated LUS findings to correlate with the severity of disease among patients with COVID-19 [22, 23] which in turn have shown to correlate with increased coagulopathy [14]. Thus, as LUS is associated with severity of disease which is associated with the COVID-19 induced coagulopathy, we hypothesize that LUS findings are associated with the development of VTE in patients hospitalized with COVID-19. Consequently, the present analysis aimed to assess the association between LUS findings and the development of VTE in a prospective cohort of hospitalized COVID-19 patients.

Methods

Population

The ECHOVID-19 study [24, 25] is a prospective cohort study of consecutively hospitalized COVID-19 patients from all hospitals in Eastern Denmark. A team of investigators visited eight predefined hospitals from two administrative regions in Denmark twice a week to recruit patients. The investigators performing the ultrasound examinations were blinded to the clinical data of all potential participants prior to including them. All eligible patients accepting participation in the study underwent echocardiography, LUS, laboratory testing, answered a questionnaire, and had their electronic health records reviewed. The LUS was performed on all patients according to a predefined protocol independently of their health status. Patients were included from March 30th to June 3rd, 2020. Inclusion criteria were hospitalized for laboratory-confirmed COVID-19, age $\geq$ 18 years, and provided written informed consent. We included patients from dedicated COVID-19 wards, but not from intensive care units. Of the 215 patients included into the ECHOVID-19 study, 12 patients did not have a LUS examination performed and were thus excluded from the present study. The study was approved by the regional ethics board and was conducted in accordance with the 2nd Helsinki Declaration. The ECHOVID-19 study is registered at Clinicaltrials.gov (NCT04377035).

Clinical data and baseline information

Methodology regarding the retrieval of clinical and baseline information is described in Supplemental Data.

Outcomes

The primary outcome was VTE, defined as incident of computed tomography confirmed pulmonary embolism ($N=15$, 7.0%) and/or ultrasound confirmed deep-vein thrombosis ($N=4$). The date of follow-up was 17th of June 2020. Information on outcome was obtained through the electronic health records.

LUS protocol and image analysis

All LUS examinations were performed by trained investigators using the portable Vivid IQ 4D Ultrasound System (GE Healthcare, Horten, Norway) using a phased array 1.7/3.3 MHz transducer in sagittal orientation. A standardized LUS protocol was employed with the imaging depth set to 18 cm with patients in a semi-recumbent position. A total of 8 LUS zones (4 on each hemithorax) were recorded for 6 s each as previously described [17]. All ultrasound images were reviewed and scored by two experienced investigators together (EP & CE).

Three different methods were examined to assess the extent of lung ultrasound findings as a sign of increased density of the lung parenchyma in study participants: (1) the total number of B-lines per patient was calculated as the sum of the maximum number of B-lines from each zone regardless of the number of missing zones, (2) a positive B-line score (interstitial syndrome) was defined as ≥ 3 B-lines in ≥ 2 zones on each hemithorax which has previously been employed in patients with acute heart failure [26] and (3) a LUS-score incorporating several LUS findings from each zone was utilized in which patients could be assigned a LUS-score between 0 and 16 points (Supplementary Table 1). Patients would receive 1 point for a LUS zone if the LUS zone had at least one of the following

findings: ≥ 3 B-lines, confluent B-lines (if a confluent B-line took up ≥ 50% of the intercostal space and could not be counted as separate B-lines at any time during the six sec. clip), subpleural consolidations or lobar consolidations with air bronchograms. If two findings or more were present in the same zone (e.g. ≥ 3 B-lines + subpleural consolidation), a patient would receive 2 points for this zone. In these main analyses, all patients were included. Figure 1 illustrates three examples of pathological LUS findings. The median number of missing zones was 0 (IQR: 0, 1) and 19.7% had ≥ 2 missing zones. To account for missing LUS zones, three sensitivity analyses were carried out. In the first sensitivity analysis, the number of missing LUS zones was included as a variable in the demographic regression model. In a second sensitivity analysis, only 6 LUS zones were used (3 on each hemithorax, excluding the lower lateral zones), and third, patients with at least one missing zone in the 8-zone model were excluded (75 patients left out of this analysis due to these criteria). The sensitivity analyses were carried out as some LUS zones (lower lateral zones) are more difficult to acquire adequate images of for LUS analysis. This is especially true for patients with severe respiratory distress. Thus, several models were used to control for the possibility of systematic missing data driving the statistical findings of the main results.

Echocardiography

The echocardiographic examinations were performed bedside with portable Vivid IQ Ultrasound Systems (GE Healthcare, Horten, Norway). All echocardiographic measurements were performed according to existing guidelines [4, 5]. A detailed description of the echocardiography analysis methods has previously been published [6]. Left ventricular ejection fraction (LVEF) was measured using the Simpson's biplane method. Pulsed-wave Doppler was used to measure early peak inflow velocity (E) in the apical four-chamber view with the sample area placed between the tips of the mitral leaflets. Early diastolic tissue Doppler velocities (e') were measured in the apical four-chamber view at the septal and lateral wall of the mitral annulus and e' was indexed to E to obtain E/e'. Tricuspid annular plane systolic excursion (TAPSE) was measured using M-mode in the apical 4-chamber projection optimized for TAPSE. Tricuspid regurgitation peak gradient was assessed with continuous-wave Doppler imaging with a sample place in the regurgitant jet.

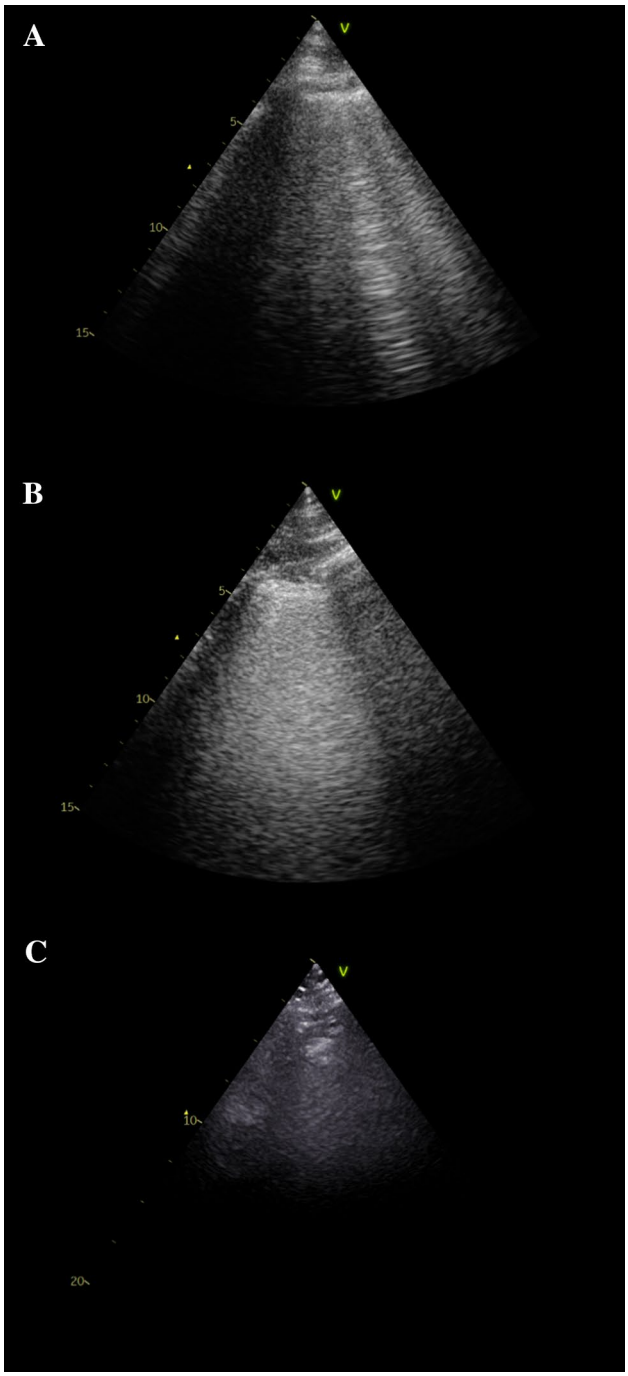

Fig. 1 Pathological LUS findings. Examples of multiple B-lines (**A**), confluent B-lines and subpleural consolidations (**B**), and lobar consolidations (**C**) assessed with LUS. *LUS* lung ultrasonography

Statistics

Baseline data (Table 1) were stratified according to the development of VTE during their hospitalization. Continuous Gaussian distributed variables are presented as mean ± standard deviations (SD) and non-Gaussian distributed variables as median with inter-quartile range (IQR). Categorical variables are presented as counts and proportions. Differences in baseline characteristics across the two groups were assessed using an extension of the Wilcoxon Rank Sum test [27] and Student's *t* test as appropriate. Univariable linear regressions were utilized

Table 1 Baseline demographic, clinical, and biochemical characteristics

	All	No VTE	VTE	*P* value
Number	203	186	17	
Baseline characteristics				
Age, years (SD)	68.6 (13.5)	68.3 (13.9)	71.6 (8.1)	0.33
Male, (%)	115 (56.7%)	107 (57.5%)	8 (47.1%)	0.40
Body mass index, kg/m^2 (SD)	26.7 (5.7)	26.9 (5.6)	25.5 (7.2)	0.35
Smoking status, (%)				
Active	12 (6.6%)	11 (6.6%)	1 (6.2%)	0.93
Previous	72 (39.3%)	65 (38.9%)	7 (43.8%)	
Never	99 (54.1%)	91 (54.5%)	8 (50.0%)	
Pack-years if smoked, (IQR)	25.0 (8.3,41.0)	20.0 (10.0,41.0)	25.0 (2.5,52.5)	0.85
Chronic obstructive lung disease, (%)	29 (14.3%)	26 (14.0%)	3 (17.6%)	0.68
Hypertension, (%)	116 (57.1%)	107 (57.5%)	9 (52.9%)	0.71
Diabetes mellitus, (%)	53 (26.4%)	50 (27.2%)	3 (17.6%)	0.39
Hyperlipidemia (%)	83 (40.9%)	79 (42.5%)	4 (23.5%)	0.13
Prevalent heart disease, (%)	11 (5.4%)	10 (5.4%)	1 (5.9%)	0.93
Peripheral artery disease, (%)	27 (13.3%)	26 (14.0%)	1 (5.9%)	0.35
History of VTE, (%)	32 (15.8%)	30 (16.2%)	2 (11.8%)	0.63
Chronic kidney disease, (%)	6 (3.0%)	5 (2.7%)	1 (5.9%)	0.46
Chronic liver disease, (%)	8 (3.9%)	8 (4.3%)	0 (0.0%)	0.38
Rheumatic disease, (%)	46 (%)	41 (%)	5 (%)	0.49
Active or previous cancer, (%)	43 (21.2%)	39 (21.0%)	4 (23.5%)	0.80
Baseline use of ACEi/ARB, (%)	42 (20.7%)	39 (21.0%)	3 (17.6%)	0.75
Baseline use of beta-blockers, (%)	18 (8.9%)	17 (9.1%)	1 (5.9%)	0.65
Baseline use of calcium channel blockers, (%)	65 (32.0%)	63 (33.9%)	2 (11.8%)	0.061
Baseline use of aldosterone antagonists, (%)	33 (16.3%)	31 (16.7%)	2 (11.8%)	0.60
Baseline use of diuretics, (%)	29 (14.3%)	26 (14.0%)	3 (17.6%)	0.68
Baseline use of NSAID, (%)	116 (57.1%)	107 (57.5%)	9 (52.9%)	0.71
Vital signs on admission				
Early warning score, (IQR)	2.0 (1.0, 4.0)	2.0 (1.0, 4.0)	2.0 (1.0, 3.0)	0.60
Systolic blood pressure, mmHg (SD)	126.2 (19.7)	126.3 (20.1)	126.1 (15.1)	0.98
Diastolic blood pressure, mmHg (SD)	73.1 (11.1)	72.7 (11.4)	76.6 (7.2)	0.99
Heart rate, beats/min (SD)	80.3 (16.6)	79.5 (16.2)	88.3 (19.1)	0.044
Temperature, Celsius (SD)	37.1 (0.7)	37.1 (0.7)	37.2 (0.6)	0.72
Respiratory rate, breaths/min (IQR)	18.0 (18.0,20.0)	19.0 (18.0,20.0)	18.0 (18.0,20.0)	0.40
Oxygen saturation, % (IQR)	95.0 (94.0,97.0)	95.0 (94.0,97.0)	95.0 (94.0,96.0)	0.61
Oxygen therapy, L/min (IQR)	1.0 (0.0, 3.0)	1.0 (0.0, 3.0)	2.0 (1.0, 3.5)	0.044
Laboratory results				
Hemoglobin, mmol/L (SD)	7.3 (1.1)	7.3 (1.1)	7.3 (1.2)	0.91
Ferritin, µg/L (SD)	1026.6 (1209.6)	1040.4 (1252.7)	868.3 (495.9)	0.62
Leucocytes, 10^9/L (IQR)	6.3 (4.6,9.0)	6.3 (4.6,8.8)	7.3 (4.7,10.0)	0.49
Lymphocytes, 10^9/L (IQR)	1.1 (0.7,1.5)	1.2 (0.7,1.6)	1.0 (0.8,1.4)	0.42
Neutrophils, 10^9/L median (IQR)	4.5 (3.1,6.7)	4.5 (3.1,6.7)	5.0 (3.5,7.2)	0.26
Procalcitonin µg/L, median (IQR)	0.2 (0.1,0.5)	0.2 (0.1,0.7)	0.2 (0.1,0.2)	0.63
CRP, mg/L (IQR)	57.5 (24.0,96.0)	58.0 (21.0,95.0)	55.0 (27.0,97.0)	0.87
eGFR, mL/min/1.73 m^2 (IQR)	86.1 (62.7,112.3)	85.0 (62.0,110.0)	98.2 (78.6,123.2)	0.094
Urea, mmol/L (IQR)	5.5 (3.8,8.6)	5.2 (3.8,8.6)	5.8 (5.4,6.7)	0.59
Hemoglobin A1c, mmol/mol (IQR)	43.0 (38.0,47.0)	43.0 (38.0,47.0)	41.5 (36.0,49.0)	0.92
HDL cholesterol, mmol/L (SD)	0.9 (0.3)	0.9 (0.3)	1.0 (0.4)	0.085
LDL cholesterol, mmol/L (SD)	1.8 (0.8)	1.8 (0.8)	2.2 (0.9)	0.13
VLDL cholesterol, mmol/L (SD)	0.8 (0.4)	0.8 (0.4)	0.7 (0.3)	0.21

Table 1 (continued)

	All	No VTE	VTE	*P* value
Total cholesterol, mmol/L mean (SD)	3.5 (1.0)	3.4 (1.0)	3.9 (1.3)	0.13
D-Dimer mg/L	1.3 (0.7, 2.5)	1.2 (0.7, 2.2)	5.0 (1.3, 6.4)	0.002
Echocardiography				
LVEF, % (SD)	57.6 (9.0)	57.6 (9.0)	57.4 (8.9)	0.95
E/e', (IQR)	8.5 (6.8, 11.9	8.5 (6.8, 12.0)	8.4 (6.4, 9.6)	0.28
TAPSE, mm (%)	2.00 (0.45)	2.00 (0.46)	2.02 (0.28)	0.86
TR gradient, mmHg (IQR)	22.6 (17.7, 27.6)	22.5 (17,7, 28.1)	23.1 (20.2, 26.8)	0.84
Lung ultrasonography				
Time from admission to LUS, days (IQR)	4.0 (2.0, 8.0)	4.0 (2.0, 8.0)	3.0 (2.0, 8.0)	0.72
Number of B-lines, (IQR)	12 (8, 18)	12 (7, 17)	17 (10, 23)	0.044
B-line score, (%)	44 (21.7%)	35 (18.8%)	9 (52.9%)	0.001
LUS-score, (IQR)	3 (1,5)	2 (1,5)	6 (2,7)	0.021
1 ≥ B-line in one zone, (%)	202 (99.5%)	185 (99.5%)	17 (100.0%)	0.76
1 ≥ confluent B-lines in one zone, (%)	38 (18.7%)	35 (18.8%)	3 (17.6%)	0.91
1 ≥ subpleural or lobar consolidation, (%)	30 (14.8%)	25 (13.4%)	5 (29.4%)	0.076

to assess the relationship between LUS-score and conventional echocardiographic left and right ventricular parameters. Cox proportional hazard regression models (univariable and multivariable) were used to assess the continuous association between total number of B-lines and subsequent VTE, B-line score and subsequent VTE, and LUS-score and subsequent VTE, respectively, excluding participants with VTE prior to LUS ($N=5$). A multivariable demographic model was adjusted for age and sex. The fully adjusted multivariable model included additional possible confounding covariates; age, sex, C-reactive protein (CRP), body mass index (BMI), D-dimer ≥ 0.50 mg/L and level of oxygen therapy (liters of O_2/minute). These covariates were chosen based on their clinical importance and their known association with VTE. A limited number of variables were utilized to prevent overfitting. Logistic regression models (univariable and multivariable) were carried out following the same procedure, not excluding VTE participants prior to LUS. Sensitivity analyses were performed, as described in the LUS section. Restricted cubic spline models were constructed using a Poisson model to visualize the association between total number of B-lines and VTE, and LUS-score and subsequent VTE, respectively. The number of knots was chosen according to the lowest Akaike information criterion. The optimal cut-off values for total number of B-lines (≥ 15) and LUS-score (≥ 5) were determined through receiver operating characteristics curves with the maximal sensitivity and specificity. STATA statistics/data analysis, SE 15.0 (StataCorp, College Station, TX, USA) was used for all data work. Statistical significance was defined as a *P* value ≤ 0.05.

Results

A total of 203 hospitalized COVID-19 patients were included in the study. The mean age was 68.6 ± 13.5 years, and 56.7% were males. The median time from hospitalization to LUS examination was 4 days (IQR: 2, 8). In total, 17 patients demonstrated VTE during hospitalization, 12 of these events were following LUS examination (median time from LUS examination to VTE was 4 days (IQR: 1, 9), while 5 events were prior to LUS examination. The median time to VTE from hospitalization was 9 days (IQR: 5, 17). Finally, participants experienced symptoms of COVID-19 median 7 days (IQR: 3, 10) prior to the day of admission.

Median LUS-score was 3 (IQR: 1, 5), median number of B-lines was 12 (IQR: 8, 18), and 44 (22%) had positive B-line score. Most participants (99.5%) had at least 1 B-line in one zone, 18.7% had at least one group of confluent B-lines in one zone, 14.8% had at least 1 subpleural or lobar consolidation, and 83.3% had a LUS-score ≥ 1. Pleural effusion was not observed in any of the included patients. Demographic, clinical, laboratory and LUS characteristics are compared between patients who developed VTE and patients who did not in Table 1. Several significant differences were observed between the two groups. Participants developing VTE were more likely to have a higher heart rate, level of oxygen therapy, and elevated D-dimer (5.0 [IQR: 1.3, 6.4] mg/L vs 1.2 [IQR: 0.7, 2.2] mg/L, $P=0.002$). Patients with VTE also had a higher number of B-lines (17 [IQR: 10, 23] vs 12 [IQR: 7, 17], $P=0.044$), more frequently positive B-line score (9 (52.9%) vs 35 (18.8%), $P=0.001$), and higher LUS-score (6 [IQR: 2, 7], vs 2 [IQR: 1, 5], $P=0.021$).

Relationship between LUS-score and echocardiographic parameters

The relationships between LUS-score and conventional echocardiographic left and right ventricular parameters were assessed with univariable linear regression models. High LUS-score was not associated with elevated TR gradient (stand. β.coefficient: 0.16, $P=0.051$), elevated E/E' (stand. β.coefficient: 0.00, $P=0.99$), reduced LVEF (stand. β.coefficient: −0.03, $P=0.74$) or reduced TAPSE (stand. β.coefficient: −0.11, $P=0.14$).

Relationship between LUS findings and VTE

Uni- and multivariable Cox and logistic regression models investigating the association between LUS findings (number of B-lines, B-line score, and LUS-score) and VTE are listed in Table 2. In univariable Cox (only VTE cases following the LUS examination) and logistic (all cases of VTE) regressions, an increasing number of B-lines, positive B-line score, and LUS-score were all significant markers of outcome.

Number of B-lines was significantly associated with higher risk of VTE in multivariable Cox regression (HR = 1.14, 95% CI (1.03, 1.26), $P=0.016$, per 1 B-line increase). This was also the case in multivariable logistic regression (OR = 1.09, 95% CI (1.01, 1.19), $P=0.038$, per 1 B-line increase). B-line score also remained a significant marker of VTE during hospitalization in both multivariable Cox and logistic regression models (Cox regression: (HR = 9.79, 95% CI (1.87, 51.35), $P=0.007$; logistic regression: OR = 6.10, 95% CI (1.69, 22.05), $P=0.006$). Finally, LUS-score remained independently associated with VTE during hospitalization in the fully adjusted models (Cox regression: HR = 1.51, 95% CI (1.10, 2.07), $P=0.010$ per 1-point increase; logistic regression: OR = 1.39, 95% CI (1.09, 1.79), $P=0.009$, per 1-point increase). The

Table 2 The relationship between VTE and LUS findings

	Cox regression (12 VTE)			Logistic regression (17 VTE)		
	HR^	95% CI	*P* value	OR*	95% CI	*P* value
B-lines per 1 increase						
Unadjusted	1.09	1.01,1.18	*0.027*	1.08	1.01,1.16	*0.030*
Demographic adjustments	1.09	1.01,1.19	*0.032*	1.09	1.01,1.17	*0.021*
Multivariable adjustments	1.14	1.03,1.26	*0.016*	1.09	1.01,1.19	*0.038*
Sensitivity analysis 1	1.08	0.99,1.18	*0.068*	1.08	1.01,1.16	*0.036*
Sensitivity analysis 2	1.11	1.00,1.23	*0.052*	1.10	1.00,1.20	*0.045*
Sensitivity analysis 3	1.08	0.99,1.78	*0.10*	1.07	0.98,1.16	*0.100*
Positive B-line score						
Unadjusted	4.2	1.33,13.32	*0.014*	4.85	1.75,13.47	*0.002*
Demographic adjustments	4.45	1.40,14.14	*0.011*	5.13	1.82,14.40	*0.002*
Multivariable adjustments	9.79	1.87,51.35	*0.007*	6.10	1.69,22.05	*0.006*
Sensitivity analysis 1	4.00	1.23,13.05	*0.022*	5.58	1.90,16.40	*0.002*
Sensitivity analysis 2	6.09	1.90,19.54	*0.002*	7.29	2.41, 22.09	*< 0.001*
Sensitivity analysis 3	5.54	1.27,24.16	*0.023*	4.37	1.28,14.86	*0.018*
LUS-score per 1 increase						
Unadjusted	1.25	1.02,1.53	*0.031*	1.28	1.05,1.55	*0.013*
Demographic adjustments	1.29	1.03,1.61	*0.024*	1.31	1.08,1.60	*0.007*
Multivariable adjustments	1.51	1.10,2.07	*0.010*	1.39	1.09,1.79	*0.009*
Sensitivity analysis 1	1.28	1.03,1.60	*0.026*	1.34	1.09,1.64	*0.005*
Sensitivity analysis 2	1.38	1.07,1.78	*0.013*	1.40	1.04,1.88	*0.029*
Sensitivity analysis 3	1.34	1.01,1.76	*0.040*	1.38	1.08,1.78	*0.011*

^HR/OR per 1-point increase

*HR/OR per 1 B-line increase

Multivariable adjustments includes the variables: sex, age, CRP, BMI, and level of oxygen therapy

Sensitivity analysis 1: multivariable adjustment with the previous included variables in addition to number of missing LUS zones

Sensitivity analysis 2: same multivariable adjustment model, number of B-lines, B-line score, and LUS-score, only assessed from 6 zones

Sensitivity analysis 3: same multivariable adjustment model, number of B-lines, B-line score, and LUS-score, only assessed from 6 zones, analysis restricted to patients without any missing zones

associations between LUS findings and VTE are illustrated in Fig. 2.

Three sensitivity analyses were conducted using the same multivariable model. B-line score and LUS-score both remained significantly associated with the outcome in all sensitivity analyses. Meanwhile, the total number of B-lines only remained significant in logistic regression sensitivity analysis 2 and 3, (Table 2).

Optimal cutoff values for total number of B-lines and LUS-score were found to be, respectively, ≥ 15 B-lines and a LUS-score ≥ 5. The cutoff value for total number of B-lines demonstrated a sensitivity of 58.8% (IQR: 32.9; 81.6) and specificity of 76.1% (69.6; 81.9). Meanwhile, a positive B-line score had a sensitivity of 52.9% (IQR: 27.8; 77) and specificity of 82.2% (IQR: 76.2; 87.3). Finally, a LUS-score ≥ 5 had a sensitivity and specificity of, respectively, 58.8% (IQR: 32.9; 81.6) and specificity of 76.1% (69.6; 81.9).

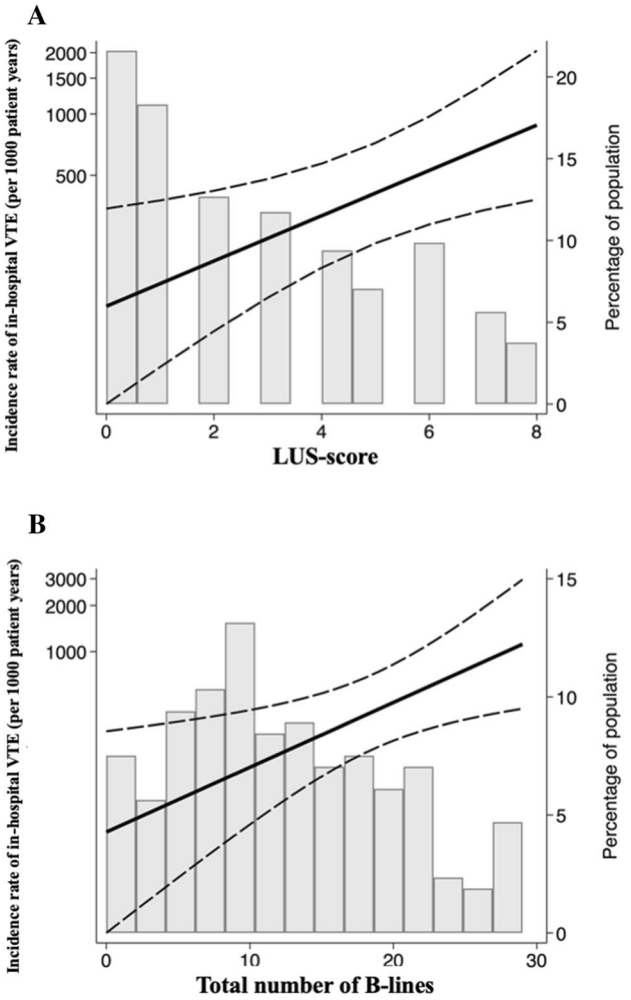

Fig. 2 Association between LUS findings and future VTE during hospitalization. Displaying the unadjusted incidence rates of VTE during hospitalization for COVID-19 (with 95% confidence intervals) for the population in relation to total number of B-lines (**A**) and LUS-score (**B**). *LUS* lung ultrasonography, *VTE* venous thromboembolic event

Discussion

The present study was designed with the objective of assessing the association between lung ultrasound findings and development of VTE in hospitalized COVID-19 patients. We particularly aimed to assess the association between LUS findings and development of VTE, as several studies have coupled the severity of the SARS-CoV-2 infection with LUS findings [22, 23], and severity of disease with dysregulated coagulation [5, 6]. To the best of our knowledge, this is the first prospective cohort study investigating the association between LUS and VTE in hospitalized patients with COVID-19. There were several important findings. First, LUS findings are common and numerous in hospitalized patients with COVID-19. Second, we found that the total number of B-lines, B-line score, and LUS-score were all significantly associated with the development of VTE during hospitalization. Finally, we did not find any significant relationship between LUS-score and conventional echocardiographic left and right ventricular parameters.

Diffuse pulmonary intravascular coagulopathy causes extravasation of fluids in the pulmonary parenchyma which can be detected by LUS as B-lines and consolidations. The viral pneumonia in itself together with a possible bacterial superinfection also causes pulmonary edema giving rise to LUS findings.

We observed that a large proportion (83.3%) of the prospectively included hospitalized COVID-19 patients in our study had ≥ 1 point on the LUS-score. In addition, 99.5% of the included patients had at least one B-line in one of the LUS zones. Several small cohort studies have investigated the presence of pathological LUS findings in patients with COVID-19 and their relation to computed tomography findings [28, 29]. One retrospective cohort study investigated whether LUS could be used to detect COVID-19 in 42 patients with COVID-19 and 24 controls without COVID-19. In that study, the B-line score had a similar sensitivity to detect COVID-19 than chest X-ray and CT and improved specificity compared to chest radiograph [29]. However, the sample size was quite small, and they did not investigate how LUS findings were associated with the development of VTE. The results described in a systematic review[28] looking at the utility of LUS in COVID-19 suggest that LUS findings are very common in COVID-19 and that their mere presence in themselves may help detect COVID-19 but are not optimal for risk stratification. Rather, it seems the cumulative amount of LUS findings (B-lines, subpleural and lobar consolidations) may be more useful as a tool for risk stratification in hospitalized patients with COVID-19.

Using only the total number of B-lines may be inadequate as we observed many cases of confluent B-lines and subpleural and lobar consolidations, making it difficult to count the number of B-lines correctly. It seems the LUS findings in COVID-19 are numerous, and we, therefore, suggest a scoring system of the kind presented in this study to account for both the number of zones affected and the possibility of multiple LUS findings per zone. A scoring system like this requires external validation, but we hope that the present study can be hypothesis generating for future LUS studies in COVID-19 and help in elucidating the prognostic potential of LUS in COVID-19.

While we in the present study have found that LUS findings were associated with VTE, the study cannot answer the question of causality as there is a potential risk of reverse causation. That is, it may be that our LUS findings are present due to VTE instead of LUS findings occurring prior to later development of VTE. We used both Cox proportional hazard regression with the outcome being restricted to VTE diagnosed after the LUS examination and logistic regression with the outcome being development of VTE during the entire hospitalization as to observe possible differences that could be due to reverse causation. However, regardless of the method used to analyze the data, LUS findings were significantly associated with the outcome in both scenarios and remained so even after adjusting for possible confounding parameters.

In the present study, we observe that the patients who developed VTE had a higher number of pathological LUS findings. What the LUS detects is increased density of the lung parenchyma most likely due to inflammation related to pneumonitis rather than lung infarction due to emboli which is usually seen as triangular, hypoechoic, subpleural consolidations. Though, diffuse pulmonary intravascular coagulopathy also adds to the increased density of the parenchyma. Our findings suggest that patients who develop VTE have a more severe pneumonitis and probably also increased endotheliitis. Thus, the risk of developing VTE is related to the clinical stage and severity of the disease and not to the extent of the pathological findings itself.

We used an 8-zone model as has previously been recommended [30] in our study. The lower lateral zones are, however, known to be difficult to obtain, in patients with prevalent respiratory failure in the semi-recumbent position. We, therefore, carried out sensitivity analyses to make sure that our results were not driven by inadequate lower lateral image clips in patients with the worst respiratory function. Still, the LUS findings remained significantly associated with the outcome in the majority of sensitivity analyses underscoring that our findings were not driven by differences in missing LUS zones. As the association between LUS findings and VTE remained significant in a 6-zone model, a lower number of zones may suffice in the clinical setting. However, further studies are needed to confirm this observation.

Strengths and limitations

This study is limited by a relatively small sample size. However, we used a multi-center design in which we included patients from all hospitals (8 hospitals) in Eastern Denmark. The multi-center design further strengthens our results as it makes our sample population much more representative of the general population. An important limitation to this study is that the observative design meant that not all participants had a CT angiography performed and thus some of the patients in the non-VTE group may actually have had an undetected VTE. As many of the patients had severe respiratory distress, the LUS examination was at times difficult to perform, especially in the lower lateral zones. Due to this, some of the patients had inadequate lower lateral zones for LUS analysis. In addition, many of the patients were too ill to collaborate to be in a seated position. All of this meant that we chose not to use a 12-zone LUS model which would otherwise have allowed us to assess the posterior zones and instead chose to only assess 8 zones (frontal and lateral zones) which limits our findings although the 8-zone model has been successfully used in several other studies [31, 32]. However, several sensitivity analyses were carried out which confirmed our findings in the primary analyses. A low-frequency probe was used for the study; however, a higher frequency probe would have improved resolution and may have aided in identifying even smaller subpleural consolidations which may have been missed. Due to the relatively small sample size, the number of events was low. However, our results remained stable in several different multivariable regression models and sensitivity models. Lastly, an important limitation is the variability in days from admission to LUS examination. A small team of investigators visiting two of the eight sites each day meant that each site was visited twice per week. Due to this approach, the median time from admission to ultrasound examination was 4 days, and as a result, a subset of our cohort probably represents a subset of COVID-19 patients requiring a longer hospital stay. This limits the generalizability of our results as they most likely reflect patients requiring longer hospital stays. The LUS investigators were experts in ultrasound and physicians without LUS experience may demonstrate poorer diagnostic performance. However, acquisition of LUS images is fairly simple and B-lines are easily recognized.

Conclusion

In hospitalized COVID-19 patients, pathological LUS findings were common, and the total number of B-lines, B-line score, and LUS-score were all associated with VTE. These

findings indicate that the LUS examination may be useful in risk stratification and the clinical management of COVID-19. These findings should be considered hypothesis generating.

Author contributions All the authors have contributed to the study in a manner that merits authorship as described in the journals' "guideline for authors".

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Data availability Data are available upon request.

Code availability Coding is available upon request.

Declarations

Conflict of interest TBS reports receiving research grants from Sanofi Pasteur and GE Healthcare, is a Steering Committee member of the Amgen financed GALACTIC-HF trial, on advisory boards for Sanofi Pasteur and Amgen, and speaker honorariums from Novartis and Sanofi Pasteur. EP has received research support from the NIH outside the submitted work, and consulting fees from scPharmaceuticals outside the submitted work. Her employer has received support from Novartis for consulting work outside the submitted work. The remaining authors have nothing to disclose in relation to the present project.

Ethics approval The study was approved by the regional ethics board and was conducted in accordance with the 2nd Helsinki Declaration. The ECHOVID-19 study is registered at Clinicaltrials.gov (**NCT04377035**).

Consent to participate Informed consent was obtained from all participants.

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Authors and Affiliations

Kristoffer Grundtvig Skaarup[1] · Mats Christian Højbjerg Lassen[1] · Caroline Espersen[1] · Jannie Nørgaard Lind[1] · Niklas Dyrby Johansen[1] · Morten Sengeløv[1] · Alia Saed Alhakak[1] · Anne Bjerg Nielsen[1] · Kirstine Ravnkilde[1] · Raphael Hauser[1] · Liv Borum Schöps[1] · Eva Holt[1] · Henning Bundgaard[2] · Christian Hassager[2] · Reza Jabbari[2] · Jørn Carlsen[2] · Ole Kirk[3] · Uffe Bodtger[4] · Matias Greve Lindholm[5] · Lothar Wiese[6] · Ole Peter Kristiansen[7] · Emil Schwarz Walsted[8] · Olav Wendelboe Nielsen[7] · Birgitte Lindegaard[9] · Niels Tønder[10] · Klaus Nielsen Jeschke[11] · Charlotte Suppli Ulrik[11] · Morten Lamberts[1] · Pradeesh Sivapalan[12] · Jannik Pallisgaard[1] · Gunnar Gislason[1] · Kasper Iversen[1] · Jens Ulrik Stæhr Jensen[12] · Morten Schou[1] · Søren Helbo Skaarup[13] · Elke Platz[14] · Tor Biering-Sørensen[1,15]

[1] Cardiovascular Non-Invasive Imaging Research Laboratory, Department of Cardiology, Herlev & Gentofte Hospital, University of Copenhagen, Niels Andersens vej 65, 2900 Hellerup, Denmark

[2] Department of Cardiology, Rigshospitalet, University of Copenhagen, Hellerup, Denmark

[3] Department of Infectious Diseases, Rigshospitalet, University of Copenhagen, Hellerup, Denmark

[4] Department of Respiratory and Internal Medicine, Næstved-Slagelse Hospital, University of Southern Denmark, Odense, Denmark

[5] Department of Cardiology, Zealand University Hospital Roskilde, University of Copenhagen, Hellerup, Denmark

[6] Department of Infectious Diseases; Zealand University Hospital Roskilde, University of Copenhagen, Hellerup, Denmark

[7] Department of Cardiology, Bispebjerg & Frederiksberg Hospital, University of Copenhagen, Hellerup, Denmark

[8] Department of Respiratory Medicine, Bispebjerg & Frederiksberg Hospital, University of Copenhagen, Hellerup, Denmark

[9] Department of Respiratory Medicine and Infectious Diseases, Nordsjællands Hospital, University of Copenhagen, Hellerup, Denmark

[10] Department of Cardiology, Nordsjællands Hospital, University of Copenhagen, Hellerup, Denmark

[11] Department of Respiratory Medicine, Amager Hvidovre Hospital, University of Copenhagen, Hellerup, Denmark

[12] Department of Medicine, Herlev & Gentofte Hospital, University of Copenhagen, Hellerup, Denmark

[13] Department of Respiratory Medicine and Allergy, Aarhus University Hospital, University of Aarhus, Aarhus, Denmark

[14] Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School, Boston, USA

[15] Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Hellerup, Denmark