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CHEST

Feasibility of a New Lung Ultrasound Protocol to Determine the Extent of Lung Injury in COVID-19 Pneumonia

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BACKGROUND: Lung ultrasound (LUS) scanning is useful to diagnose and assess the severity of pulmonary lesions during COVID-19-related ARDS (CoARDS). A conventional LUS score is proposed to measure the loss of aeration during CoARDS. However, this score was validated during the pre-COVID-19 era in patients with ARDS in the ICU and does not consider the differences with CoARDS. An alternative LUS method is based on grading the percentage of extension of the typical signs of COVID-19 pneumonia on the lung surface (LUSext).

RESEARCH QUESTION: Is LUSext feasible in patients with COVID-19 at the onset of disease, and does it correlate with the volumetric measure of severity of COVID-19 pneumonia lesions at CT scan (CTvol)?

STUDY DESIGN AND METHODS: This observational study enrolled a convenience sampling of patients in the ED with confirmed COVID-19 whose condition demonstrated pneumonia at bedside LUS and CT scan. LUSext was visually quantified. All CT scan studies were analyzed retrospectively by a specifically designed software to calculate the CTvol. The correlation between LUSext and CTvol, and the correlations of each score with Pao_2/Fio_2 ratio were calculated.

RESULTS: We analyzed data from 179 patients. Feasibility of LUSext was 100%. Time to perform LUS scan was 5 ± 1.5 mins. LUSext and CTvol were correlated positively ($R = 0.67$; $P < .0001$). Both LUSext and CTvol showed negative correlation with Pao_2/Fio_2 ratio ($R = -0.66$ and $R = -0.54$; $P < .0001$, respectively).

INTERPRETATION: LUSext is a valid measure of the severity of the lesions when compared with the CT scan. Not only are LUSext and CTvol correlated, but they also have similar inverse correlation with the severity of respiratory failure. LUSext is a practical and simple bedside measure of the severity of pneumonia in CoARDS, whose clinical and prognostic impact need to be investigated further. CHEST 2023; 163(1):176-184

KEY WORDS: ARDS; COVID-19 pneumonia; CT volumetry; interstitial pneumonia; lung ultrasound; lung ultrasound scoring

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ABBREVIATIONS: CoARDS = corona virus-related ARDS; CTvol = CT volumetric scoring; GGO = ground glass opacities; LUS = lung ultrasound scanning; LUSext = lung ultrasound extension scoring; P/F = Pao_2/Fio_2 ratio; RT-PCR = reverse transcriptase-polymerase chain reaction

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Take-home Points

Study Question: Is a simplified lung ultrasound scoring, based on the eyeball estimation of the extension of pulmonary lesions (LUSext), accurate to assess the severity of lung injury in COVID-19 pneumonia?

Results: In patients with first diagnosis of COVID-19 pneumonia, LUSext correlated positively with the volumetric measure of lung lesions at CT scan ($R = 0.67$; $P < .0001$) and showed a negative correlation with the Pa_{O_2}/F_{IO_2} ratio ($R = -0.66$; $P < .0001$).

Interpretation: A simple eyeball estimation of the percentage of extension on the chest surface of the typical sonographic lesions can be used to measure the lung damage during the first diagnostic approach to COVID-19 pneumonia.

Chest imaging is strategic not only in the initial diagnosis of COVID-19-associated pneumonia but also to evaluate and monitor its severity. The diagnostic role of lung ultrasound scanning (LUS) has been explored widely and compared with confirmation by CT scan and reverse transcriptase-polymerase chain reaction (RT-PCR).[1-3] LUS diagnosis of COVID-19 pneumonia is based on the recognition of typical interstitial and parenchymal signs, together with the careful evaluation of their distribution on the lung surface and combination with clinical phenotypes.[1,2]

Together with the first diagnosis, the LUS quantification of the severity of COVID-19 pneumonia might be of great use in the management of the disease and its prognostication. The advantage of the use of LUS is based on its high feasibility and the possibility to repeat the examination at the bedside without the necessity to move the patient, thus reducing the possibility of intrahospital cross infection during a pandemic surge.

LUS is a surface imaging technique, quite limited in the evaluation of lesions that do not abut the lung periphery.[4,5] However, COVID-19 typically affects mainly the lung periphery and is characterized by typical lesions alternating with spared areas. Thus, a possibility to assess the severity of COVID-19 pneumonia is to assign a LUS score based on the visual estimation of the percentage of extension of the typical lesions on the pulmonary surface (LUSext).

Our hypothesis is that the intrinsic characteristics of COVID-19 pneumonia makes LUSext a reliable tool in the assessment of the severity of the lung damage during the first approach to the disease. The primary aim of our study is to investigate the correlation between the LUSext score and the volumetric assessment of pulmonary lesions calculated by CT scan (CTvol) in patients with COVID-19 pneumonia. A secondary aim is to evaluate whether the severity of COVID-19 pneumonia assessed by LUSext and CTvol correlates with the objective measure of respiratory failure.

Study Design and Methods

Temporal Framework

We performed an observational cross-sectional single-center study on a convenience sampling of patients with confirmed first diagnosis of COVID-19 pneumonia. Patients were enrolled in the ED of San Luigi Gonzaga University Hospital during the first wave (March to May 2020) and the second wave (October 2020 to February 2021) of the COVID-19 pandemic.

Selection of the Population

During the study period in our ED, all patients suspected of COVID-19 were examined by LUS at their first visit. Consecutive patients for whom CT scans were performed immediately after LUS for clinical reasons independent from the study protocol, were considered eligible. Only adult patients with final demonstration of acute COVID-19 pneumonia with both positive chest imaging and positive RT-PCR were selected. Based on a convenience sampling, those patients who were examined for LUSext scoring by specifically trained operators entered the analysis. Information about symptoms of presentation, the timing of symptoms onset, and first bedside clinical data, which included the Pa_{O_2}/F_{IO_2} ratio (P/F), was recorded systematically. Patients were grouped in three different clinical phenotypes at presentation, according to a protocol previously validated and based on the presence of preexisting chronic cardiac or respiratory diseases (mixed phenotype) and on the presence (severe phenotype) or absence (mild phenotype) of signs and/or symptoms of respiratory failure.[1,3] The list of significant chronic conditions included severe COPD, pulmonary fibrosis, lung cancer, heart failure, or cor pulmonale. Respiratory failure was determined according to the presence of dyspnea, either objective or self-reported, and/or desaturation after walking and/or demonstration of P/F $<$ 300 mm Hg. The local Ethical Committee approved the protocol of this study (Registro di Protocollo Generale n°2840 - 210221).

Lung Ultrasound Scan

A complete LUS examination was performed at presentation on the anterior, lateral, and posterior chest, as previously described.[1,2,6] LUSext was performed by operators specifically trained in the study protocol. Commercially available ultrasound equipment (Mindray TE7; Esaote MyLabSeven) with convex transducers (3.5 to 6.0 MHz) were used. The focus was placed at the height of the pleural line. Depth was set at approximately 8 to 10 cm, according to patient's size. Gain was regulated to optimize the whole image. The sonographers were ED clinicians, with documented experience in using LUS in emergency and critical care. During the examination, the LUS operator was blind to the result of CT scan and RT-PCR

test, but not to the patients' clinical condition. LUS was performed immediately at presentation whenever possible and always before the result of the CT scan and the RT-PCR test. Time for LUS examination was recorded and reported on the data sheet by the same operator.

LUS Diagnosis: Each LUS examination was classified according to standardized, mutually exclusive patterns, already described and validated in an international multicenter study.[3] Only patients whose condition showed the following two positive patterns were considered for the study analysis:

High Probability Pattern: Typical LUS pattern of COVID-19 pneumonia has bilateral and multifocal clusters of separated or coalescent B-lines, large hyperechoic bands (light beams), multifocal peripheral consolidations, regular and irregular pleural lines, with or without large consolidations. These clusters should appear in a patchy distribution, abruptly alternating with normal A-lines patterns ("spared areas").

Intermediate Probability Pattern: Less typical pattern includes unilateral isolated clusters of B-lines and light beam or focal multiple B-lines, with or without small peripheral consolidations.

LUS Extension Score (LUSext): A measure of the superficial extension on the chest wall of the typical COVID-19 LUS signs was calculated during the examination. The visual estimate of the percentage of extension of the lesions was reported by the operator directly at bedside. The examination was performed by recording a video of the following four areas per side (Fig 1): (1) anterior chest in longitudinal scan, (2) lateral chest in longitudinal scan, (3) posterior chest paravertebral in longitudinal scan, and (4) posterior chest below the scapula in oblique scan. In each area, it is possible to examine a variable number of intercostal spaces; usually, there are approximately four spaces in areas 1, 2 and 4, and six or more spaces in area 3. We assigned the score based on the visual extension of lesions in fixed percentages, assigning 0%, 25%, 50%, 75%, or 100% depending on the number of intercostal spaces that show the pathologic signs (Fig 2). We considered the following typical signs of COVID-19 pneumonia to assign the LUS scoring: multiple B-lines separated and coalescent, the "light beam," small peripheral consolidations with irregularity of the pleural line, and large consolidations.[3] Absence of any pathologic sign was assigned as 0%. The percentages assigned to each area were then summed and divided by the total 8 areas. This simple technique allows a rough estimate of the extension of the typical COVID-19 lesions on the lung surface, without the necessity to differentiate B-lines, consolidations, and pleural line characteristics. A demonstrative case is added as supplementary material (e-Video 1-9 and e-Fig E-1).

CT Scan

CT examinations were acquired by 64- or 128-bank CT machine (Philips Ingenuity and GE) by volumetric chest scans (slice thickness 1.25 mm; slice interval 1 mm) with parenchymal lung retro-reconstruction algorithm. Only patients with clinical indication to CT scan, which was decided independently by the physician in charge, were moved to the radiology unit inside the ED to perform the examination. The patients were in supine and head-first position and received scanning with breath held. The following parameters were used: 10 kV; 100 mAs real-time adaptive control; layer thickness 1 to 2.5 mm; pitch, 1 to 1.5; matrix, 512 × 512. All images were transmitted to the postprocessing workstation and reconstructed with the use of high-resolution and conventional algorithms. Each study was read and interpreted by two expert radiologists with long-standing experience in chest imaging.

CT Scan Diagnosis: Signs and nomenclature of CT scan were those recommended for COVID-19 and reported in European and North American societal recommendations.[7,8] Specifically, we evaluated only patients with confirmation of COVID-19 pneumonia that was supported by the visualization of one of the two CT scan positive readings: (1) "typical appearance" in the presence of peripheral, bilateral, multifocal ground glass opacities (GGO) with or without consolidation or visible lines ("crazy-paving") and (2) "indeterminate appearance" in the presence of multifocal perihilar or unilateral GGO with or without consolidation or very small GGO nonrounded or nonperipheral.

CT Scan Volumetric Score: All acquired images were processed retrospectively through a semiautomated external software (Thoracic Vcar; General Electric) to quantify the percentage of aerated, GGO, and consolidated lung parenchyma.[9-11] To this end, densitometric thresholds were identified to differentiate these entities: (1) 700 Hounsfield units to differentiate aerated lung from GGO and (2) 280 Hounsfield units to differentiate GGO from consolidated lung. These thresholds were identified based on a method validated in literature and readjusted according to the judgment of the experienced radiologist.[9] CT scans were segmented semiautomatically by the software and then refined by the experienced radiologist as needed.

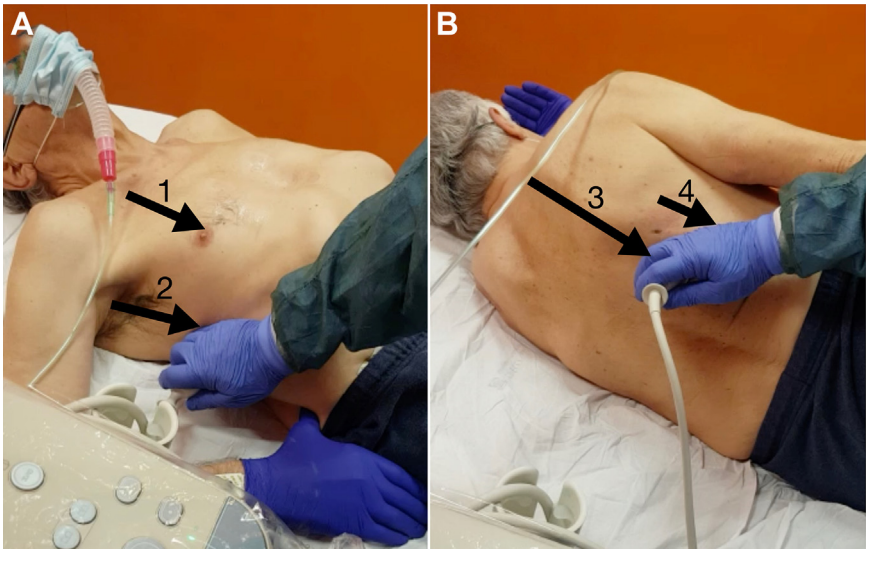

Figure 1 – *A and B, The four chest areas that were examined to assess the extension of the pulmonary lesions in patients with COVID-19 pneumonia. A, Patient is first placed in the supine position: area 1 is scanned longitudinally between the sternum and the anterior axillary line; area 2 is scanned longitudinally between the anterior axillary line and the posterior axillary line. B, Patient is then turned in the lateral decubitus: area 3 is scanned longitudinally between the spine and the medial margin of the scapula; area 4 is scanned in oblique (along the intercostal spaces) below the inferior margin of the scapula. The same procedure is then repeated on the other side.*

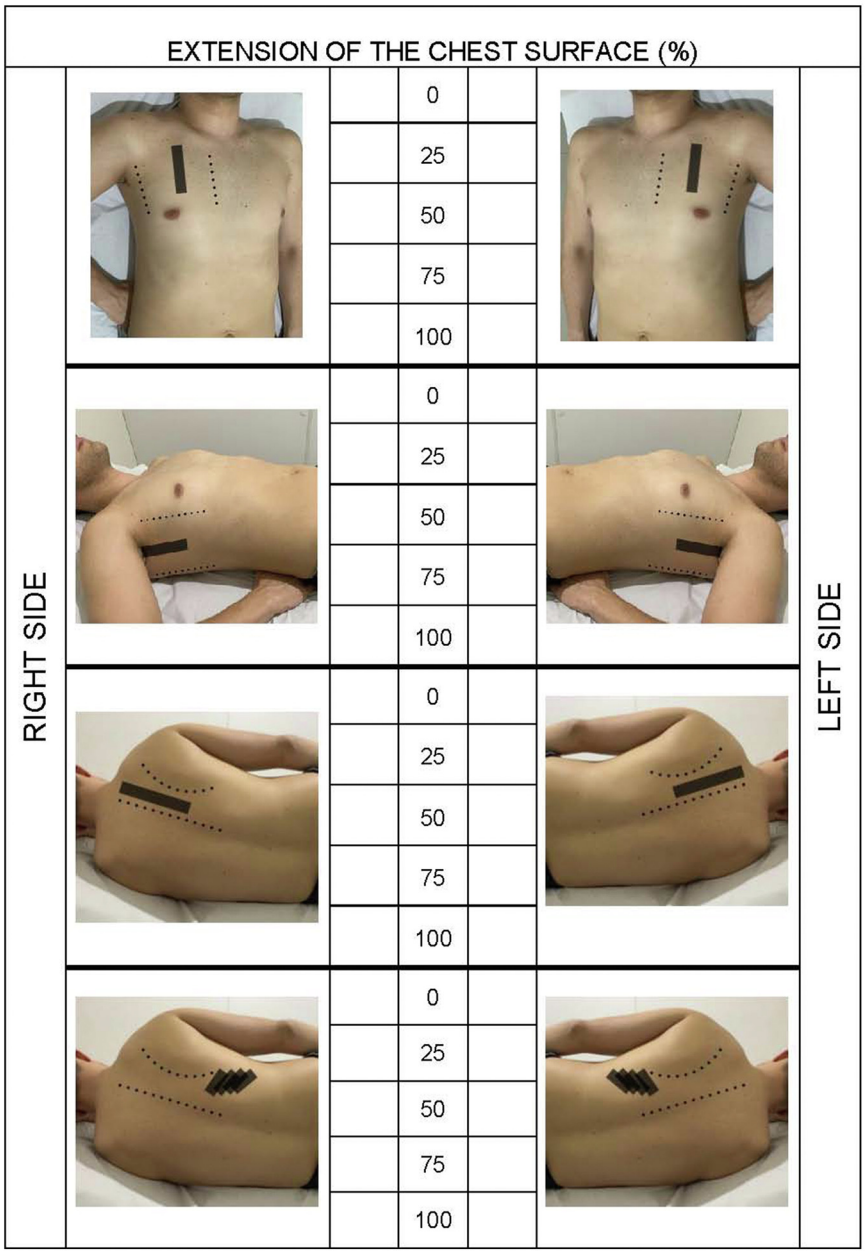

Figure 2 – *The scheme for collection of the data on the visual extension in percentage of the COVID-19 pulmonary lesions, visible by lung ultrasound examination on the chest wall. Each area is examined, and a percentage of 0-25-50-75-100% is assigned visually. The final score in percentage is given by the sum of the percentage of each area divided for the total of eight scans.*

The software automatically eliminates the airways down to the segmental branches. The vascular volume, estimated to be approximately 3%, was subtracted manually from the percentage of consolidated parenchyma. CT scans with significant motion artifacts and all those performed in patients with significant prior pulmonary alterations (severe interstitial disease, marked emphysema, lung cancer, fibrothorax) and major thoracic deformities were excluded.

RT-PCR Swab Test

The diagnosis of COVID-19 was confirmed by an RT-PCR nasal-pharyngeal and/or bronchial swab (BD SARS-CoV-2 Reagents for BD MAX System, Becton, Dickinson and Company). In pretriage, a hand-reading Rapid Antigenic Test (COVID-19 Ag Rapid Test Device; Abbott Panbio) or a facilitated reading (LumiraDx SARS-CoV-2 Ag test; LumiraDx) was performed to guide the first allocation of the patients in different areas of the ED. However, the infection was confirmed only after the RT-PCR swab detailed earlier.

Statistical Analysis

Data are expressed as mean ± SD. Normality of data was checked with the use of the Q-Q plot evaluation and Shapiro-Wilk test. Spearman rank correlation was used to examine the significance of correlation between variables. Durbin-Watson Test was used to rule out autocorrelation of residuals, and Breusch-Pagan Test was used to evaluate heteroscedasticity of residuals variance. Linear regressions were performed where appropriate. All analyses were calculated with R Studio.

Results

Patients

We analyzed by convenience sampling LUSext and CTvol of 179 patients with confirmed COVID-19 pneumonia. Sampling was determined by the availability of the personnel trained in LUSext. Figure 3 shows the patients' flow of the study; Table 1 shows the patients' characteristics.

LUS and CT Scan Diagnoses

In all patients, LUS was performed to assign the probability of COVID-19 pneumonia and to calculate LUSext. The feasibility of LUS was 100%. Average time spent for the entire LUS examination and the calculation of the LUSext score was 5 ± 1.5 min. In 12 patients (7%) the LUS diagnosis for COVID-19 pneumonia was intermediate probability and high probability in 167 patients (93%). CT scan was indeterminate in six patients (3%) and typical in 173 patients (97%). In six patients LUS intermediate probability corresponded to typical CT scan. The other six LUS diagnoses of intermediate probability corresponded to indeterminate at CT scan. All the 167 LUS high probability were typical at CT scan.

LUSext, CTvol and P/F

LUSext ranged from 3.125% to 78.125%, and CTvol ranged from 6.219% to 91.533%. A statistically significant correlation was found between LUSext and CTvol (R [Spearman Rho] = 0.67; $P < .0001$) (Fig 4A), which pointed to a good accordance between the extension of the lung lesions on the chest surface and the volume of the lung injuries in COVID-19 pneumonia. Regarding the adequacy of all the necessary statistical criteria, we calculated the linear regression between LUSext and CTvol (R-Square = 0.52), which suggests that the extensions visualized by LUS can be linked adequately through a linear relationship to the volumes obtained by CT scans. A statistically significant inverse correlation was found between LUSext and P/F values ($R = -0.66$; $P < .0001$) (Fig 4B), and between CTvol and P/F values ($R = -0.54$; $P < .0001$) (Fig 4C). We analyzed data from a subgroup of patients with more severe respiratory failure at presentation, which was selected for P/F value below 300 mm Hg (Table 1). In this subgroup the correlation between LUSext and CTvol was still significant ($R = 0.58$; $P < .0001$; and R-Square 0.41) (Fig 4D).

Discussion

Our data show that, in patients with acute onset of COVID-19 pneumonia, the LUS score based on the estimate of the LUSext is correlated positively with the CTvol. Moreover, both LUSext score and CTvol are correlated inversely to the main clinical index of respiratory function, the P/F values. In the subgroup of patients with more severe pneumonia who presented with objective respiratory failure characterized by low P/F values, LUSext and CTvol showed a similar good positive correlation, even in a condition of more complicated, extended, and mixed lung lesions. Thus, LUS performed as well as CT scan in terms of determining severity of COVID-19 lung lesions.

LUS is limited strongly in the visualization of the lung lesions because ultrasound scanning can explore only the periphery of the lung. Indeed, the alveolar air represents a barrier to the visualization of the lung parenchyma. However, it is well-acknowledged that, when the air content is impaired and the density of the lung is increased in the periphery, LUS visualizes with high

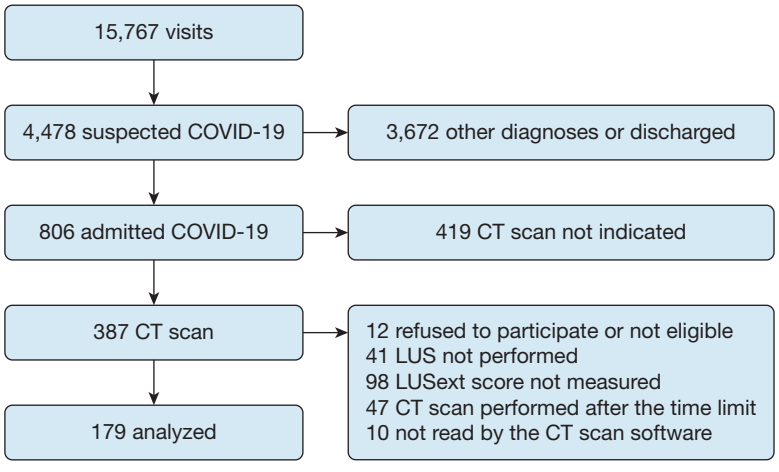

Figure 3 – *The flow diagram shows the flow of participants to our study. In the group of 98 patients who did not perform lung ultrasound extension scoring, six patients had an alternative lung ultrasound diagnosis. LUS = lung ultrasound scan; LUSext = lung ultrasound extension score.*

TABLE 1] Patient Characteristics

Characteristic	All Patients (N = 179)	Patients with Pao_2/Fio_2 Ratio < 300 mm Hg (N = 114)
Age, y	66 ± 14	69 ± 13
Male/female, No.	111/68	74/40
Onset of symptoms, d	7.7 ± 4	7.8 ± 3.7
Clinical phenotype, No. (%)		
Severe	126 (70)	108 (95)
Mild	47 (26)	0
Mixed	6 (3)	6 (5)
Lung ultrasound scan pattern, No. (%)		
Intermediate	12 (7)	2 (1.8)
High	167 (93)	112 (98.2)
CT scan appearance, No. (%)		
Indeterminate	6 (3)	0
Typical	173 (97)	114 (100)
Pao_2/Fio_2 ratio, mm Hg	277 ± 83	228 ± 54
Lung ultrasound extension score, %	36.4 ± 17.8	43.5 ± 16.5
CT volumetry, %	35.0 ± 17.0	41.0 ± 16.8
Time for lung ultrasound scan, min	5.1 ± 1.5	5.2 ± 1.5

sensitivity the typical interstitial and consolidative patterns.[12] In most pulmonary conditions, particularly in emergency situations, these LUS ultrasound patterns in combination with the available clinical information, allow for accurate finalization of the diagnostic process with high specificity. For instance, the high sensitivity and specificity of LUS are well-suited for the early diagnostic workup of acute undifferentiated respiratory failure.[13]

During the COVID-19 pandemic, LUS demonstrated a high diagnostic accuracy in the diagnosis of interstitial pneumonia related to the Sars-Cov-2 infection.[3,14] LUS can visualize the early alterations of the disease, including the GGO and the consolidations observed by CT scan in the lung periphery of patients with COVID-19 pneumonia.[15] Many authors also hypothesized a role in the quantification of lung damage during the acute phase of COVID-19.[16-19] The method of LUS scoring that has been advocated for grading the severity of lung involvement in COVID-19 pneumonia was investigated and validated in the pre-COVID era on patients with classic ARDS who received invasive ventilation.[20-22] This conventional sonographic score is based on the assignment of three grades of incremental loss of aeration through the recognition of signs of progression from separated and coalescent B-lines to consolidation, on 12 anterior, lateral, and posterior chest areas.

COVID-19 pneumonia complicated by severe respiratory failure can be included fully in the modern syndromic definition of "corona virus-related ARDS" (CoARDS). However, CoARDS presents peculiar characteristics. Indeed, evolution of the disease, histopathologic condition, extension of the lung damage, and even strategies of ventilatory support are different between ARDS and CoARDS.[23] For instance, a typical characteristic of CoARDS is the significant mismatch between the severity of the lung damage and the respiratory condition of the patient, which is not usual in both respiratory and extra-respiratory ARDS.[24] Moreover, although in ARDS, there is clear evidence that the LUS scoring is useful to guide treatment, to date, there is no robust demonstration that the severity of the lung damage assessed by LUS in CoARDS may be used in practice to predict the evolution of COVID-19 pneumonia and guide management with different ventilatory strategies.

The conventional LUS score is based on the differentiation between separated B-lines, coalescent B-lines, and consolidations. This task can be technically difficult to perform at bedside in patients with COVID-19. Assigning different grades to B-lines and consolidations may become a complicated task in a condition that, by definition, is characterized by clusters of interstitial signs and consolidations abruptly alternating with spared areas in a patchy distribution. Moreover, in a condition of tight mix of various LUS signs, the differentiation

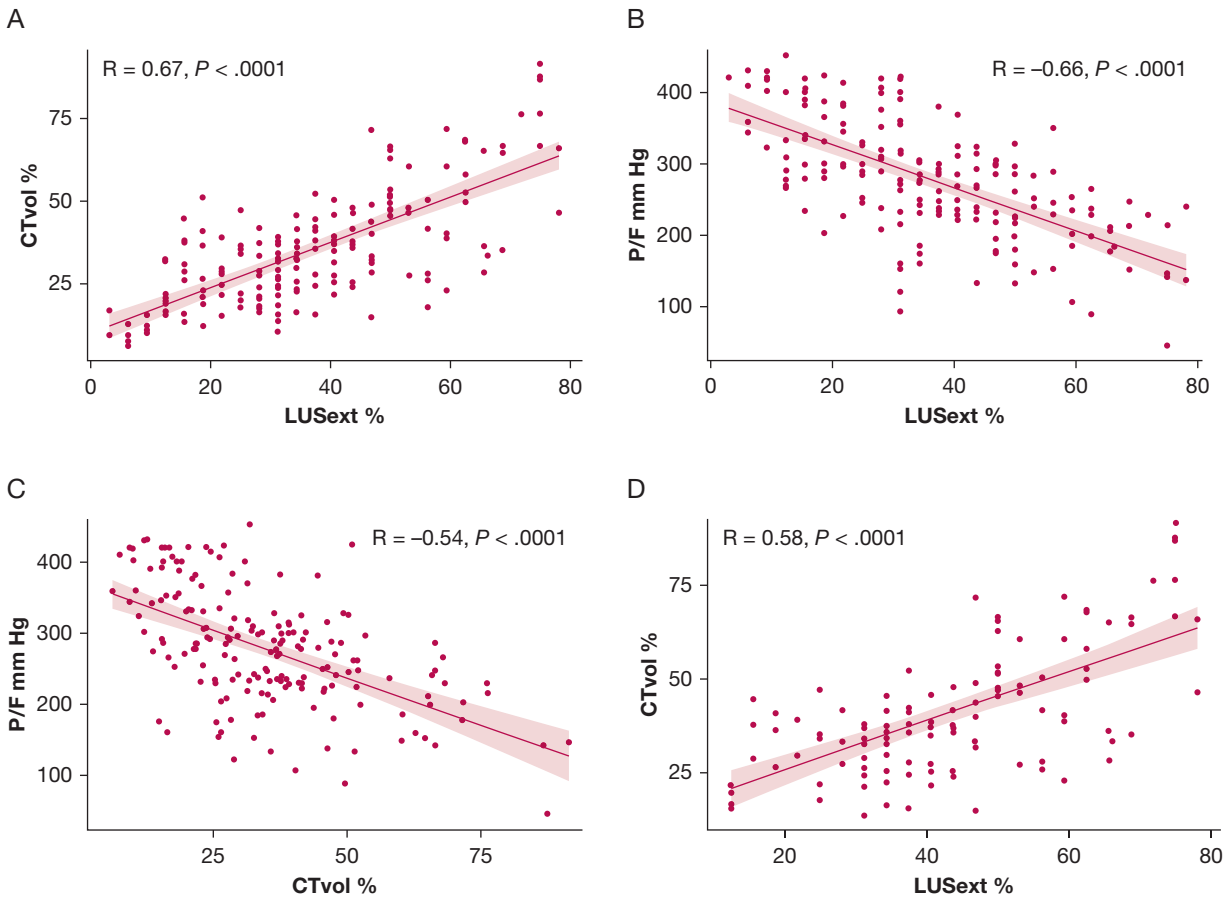

Figure 4 – *A-D, Correlations between A, lung ultrasound extension score and the CT scan volumetry of pulmonary lesions; B, lung ultrasound extension score and Pao_2/Fio_2 ratio; and C, CT scan volumetry of pulmonary lesions and Pao_2/Fio_2 ratio, in 179 patients with COVID-19 pneumonia. D, Correlations between lung ultrasound extension score and the CT scan volumetry of pulmonary lesions in a subgroup of 114 patients with Pao_2/Fio_2 ratio <300 mm Hg at presentation. CTvol = CT scan volumetry; LUSext = lung ultrasound extension score; P/F = Pao_2/Fio_2 ratio.*

between B-lines and consolidations might lose its importance as an indicator of different degrees of lung aeration.

Thus, in the opinion of these authors, the complexity of the technique and the physiopathologic mismatch between lesions and function are limitations to the practical application of the conventional LUS scoring in COVID-19. Considering these limitations, we theorized a different LUS approach to quantify the lung damage in COVID-19 pneumonia. The proposed approach is based on the estimation of the extension of the lesions and not on grading lung aeration. The extension of pneumonia on the chest surface seems to be a more appropriate index for the evaluation of the characteristics of this new disease. The new LUSext scoring does not need to differentiate different degrees of aeration and is based on a gross visual estimation of extension of lesions, which makes this new technique more immediate and easier to be performed at bedside.

The main limitation of our study is the lack of demonstration of the usefulness and practical implication of LUSext. For instance, we did not collect data on the outcomes and their correlation with LUSext. However, the only aim of the present study was to investigate the feasibility of LUSext and the correlation between LUSext and CTvol in patients with an initial diagnosis of COVID-19 pneumonia. It is highly probable that LUSext represents just a picture of the disease at a given moment that probably, like other chest imaging and respiratory scorings, is not useful in practice to predict evolution and prognosis in severe cases of COVID-19. In our experience, this new LUS scoring might indicate the necessity for a more careful follow-up and hospitalization in patients with a borderline condition and without signs of respiratory failure who show more severe grades of extension of pneumonia. This hypothesis, together with other possible practical applications of LUSext, need a scientific demonstration by future observational trials and validating studies.

Another limitation is the lack of assessment of interoperator variability of LUSext. This may be considered particularly important given the "eyeball" characteristic of our method. However, the aim of this study was to introduce a new method and assess the correlation with volumetric CT scan. Indeed, the conventional aeration LUS scoring has some subjectivity that was not assessed in the original introductory study.[21] During a COVID-19 pandemic surge assessment of variability may be particularly challenging for the necessity to limit exposure of the operators.

During the study period, more than one-half of the eligible patients who received a CT scan were not analyzed (Fig 3). Indeed, the enrollment was on a convenience sampling based on the availability of the trained operator. However, selection was completely random, and patients were enrolled at any time of the day during the pandemic surge in the ED. Moreover, enrolling only patients with indication to CT scan may have influenced selection of more severe grade of lung injury. Potentially, this might limit generalizability of our conclusions to minor forms of COVID-19.

A minor limitation is the use of P/F calculated in spontaneous breathing as a measure of respiratory failure. Indeed, it is well-acknowledged that the F_{IO_2} percentage extracted during administration of oxygen flux in mask or calculated in room air is less accurate than the true F_{IO_2} calculated in intubated patients. However, P/F during spontaneous breathing is indicative of the respiratory status during the initial evaluation and represents the important parameter to decide ventilatory treatment. Moreover, most of the patients who presented to the ED during the COVID-19 pandemic outbreak did not need intubation.

Finally, in our study a particularly savvy group of clinicians performed the LUS examinations. This may raise concern about the possibility that expertise affects accuracy. However, a previous international multicenter study demonstrated that LUS in COVID-19 may be performed by several operators with different levels of expertise maintaining high accuracy and low variability.[3]

Interpretation

Our new LUS score that is based on the eyeball estimation of the percentage of extension on the chest surface of the signs of COVID-19 pneumonia correlates positively with the CTvol scan and inversely with the P/F at the onset of the disease. This new simplified and practical LUS scoring approach is well-suited to grade the pulmonary damage of this new disease at presentation.

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Author contributions: G. V. takes the responsibility for the content of the manuscript, including the data and analysis. G. V. designed the study; G. V., T. F., and G. M. contributed equally to perform the ultrasound examinations; L. C., G. S., R. S., A. P., and D. B. performed and interpreted the CT scan studies; G. S., A. P., and D. B. performed the volumetric calculations of the CT scan examinations; R. S. is responsible for the statistical analysis; G. V., T. F., L. C., R. S., and G. M. had full access to all the data in the study; G. V., T. F., L. C., and G. M. contributed substantially to the writing of the manuscript; all authors read and approved the final version of the manuscript. We thank all the patients and hospital staff who agreed and contributed to the feasibility of the study.

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Additional information: The e-Figures and Videos are available online under "Supplementary Data."

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