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Utility of lung ultrasound in selecting older patients with hyperinflammatory phase in COVID-19 pneumonia. A monocentric, cross-sectional pilot study

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Background. Cytokine dysregulation plays a critical role in COVID-19, and its timely recognition is pivotal for a favourable outcome, typically in the oldest patient. Lung ultrasound (LUS) has been proven to be an easy-to-perform, accurate tool for detecting COVID-19 pneumonia. The current study aimed to evaluate the relationship between inflammatory markers and pulmonary injury assessed by LUS in older patients with COVID-19.
Methods. We consecutively evaluated older patients (age ≥ 65 years) hospitalized for COVID-19 pneumonia in our tertiary care hospital. All the patients underwent LUS, physical examination, and blood tests. LUS score for monitoring aeration, based on the number of B-lines for each scanned zone was assessed. Kendall's Correlation was calculated to verify the relationship between LUS and inflammation markers. A 7.5 mg/dl Hs-CRP cut-off was set to define the "hyper-inflammation" state. Finally, a receiver operating curve (ROC) was evaluated to define a cytokine storm – LUS-defined cut-off.
Results. Overall, 65 older patients [mean (SD), 82.0 (6.9) years] were included in the analysis. LUS score was related inversely to PaO_2/FiO_2 ratio at admission (tau -0.29, $p < 0.01$) and nadir (tau -0.21, $p < 0.01$), and positively to Hs-CRP (tau 0.35, $p < 0.001$). An indexed LUS score higher than 0.8 was highly predictive of cytokine storm (AUROC 0.78, $p < 0.001$; Sensitivity 86%, Specificity 68%).
Conclusions. Lung involvement evaluated by LUS correlates directly with inflammatory markers and inversely with PaO_2/ FiO_2 ratio. LUS values qualified as an independent predictor of cytokine storm, and a score greater than 0.8 is the most predictive cut-off.

Key word: infections and neoplasm, immune system and inflammation, respiratory tract

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INTRODUCTION

Since its appearance in Wuhan (China) in December 2019, SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) infection has rapidly spread around the world [1]. Although the advent of vaccination has markedly reduced the incidence of severe COVID-19, its clinical presentation remains heterogeneous, particularly in those unvaccinated [2,3]. Patients

may be asymptomatic or show a mild form of upper respiratory infection [4,5]; otherwise, SARS-CoV-2 may present a severe form of interstitial pneumonia requiring hospitalization, which can subsequently progress, due to an abnormal immune response (cytokine storm), into acute respiratory distress syndrome (ARDS) [6,7]. As a fact [8-10] the particular vulnerability to COVID-19 observed in geriatric patients seems to be linked to an excessive and inappropriate inflammatory response secondary to abnormal production of cytokines in a complex context of intrinsic compromise of the host's immune system. In severe forms of COVID-19, there is a marked increase in both inflammation indexes and serum levels of cytokines [11,12]. This inflammatory state represents an adverse prognostic factor for the need for mechanical ventilation, possible development of ARDS, and death [4,12-15]; therefore, it is not surprising that the majority of study trials on COVID-19 have focused on developing treatments able to influence this cytokine dysregulation.

Current guidelines of the Infectious Diseases Society of America on COVID-19 recommend the implementation of IL-6 inhibitors in addition to corticosteroids in severely or critically ill adult patients [16]; however, correct timing for immunomodulators initiation remains of pivotal importance and their assumption in early phases of COVID-19 has been associated with poor outcomes, particularly in older patients [17]. In this regard, lung ultrasound (LUS) has proved to be a valid, rapid, and effective tool in the early diagnostic and therapeutic approach of patients with COVID-19 disease [18,19]. More in-depth, LUS appears to be a valuable tool for estimating early pulmonary involvement during the paucisymptomatic phase of the disease and consequently plays a crucial role in the therapeutic strategy aimed at preventing cytokine storms, especially in geriatric patients.

The correlation of LUS score with the clinical conditions at admission, the time elapsed from the onset of symptoms, and some simple routine blood tests seem to allow a better and faster characterization of disease severity at the first evaluation in ED [20].

However, few studies have investigated the direct correlation of ultrasound scores with levels of inflammation markers, and few data are currently available regarding the geriatric population.

Given these premises, this study aims to evaluate the relationship between inflammatory markers and pulmonary involvement assessed by ultrasound scores in geriatric patients suffering from COVID-19 pneumonia. The secondary endpoint is to define a LUS score cut-off compatible with the hyperinflammatory state that can lead to the cytokine storm.

METHODS

In this prospective single-center observational study, we consecutively enrolled all patients aged 65 or older admitted between November 1 and December 20, 2020 to the Acute Geriatrics Unit of our tertiary care hospital. All patients underwent thorough clinical/anamnestic evaluation and blood exams. Exclusion criteria were: (i) critical illness requiring invasive ventilation at admission; (ii) presence of concomitant pulmonary oedema at admission; (iii) pre-existing interstitial diseases. Patients underwent a diagnostic examination with bedside chest echo performed on 6-8 fields per hemithorax by calculating total LUS score as modified by Bouhemad [21]. A convex and a linear covered probe, 3.5 to 7.5 MHz (Esaote Medical System), were used for chest ultrasound examination. For patients with severe mobility limitations, two operators were concomitantly involved, according to current guidelines [22]. As for the scanning scheme, in the absence of a standardized score for COVID-19 patients, we used a previously validated score [21] namely LUS score for monitoring aeration, ranging from 0 to 3 points in each of the 12 or 16 scanned zones. Score 0: predominant A-lines or less than 3 separated B-lines. Score 1: at least 3 B-lines or coalescent B-lines occupying ≤ 50% of the screen without a clearly irregular pleural line. Score 1p: at least 3 B-lines or coalescent B-lines occupying ≤ 50% of the screen with a clearly irregular pleural line. Score 2: coalescent B-lines occupying > 50% of the screen without a clearly irregular pleural line. Score 2p: coalescent B-lines occupying > 50% of the screen with a clearly irregular pleural line. Score 3: large consolidations (at least > 1 cm). The final score is obtained by summing up the scores of each area; moreover, an indexed score was obtained by dividing the final score for the total of zone recorded (12 or 16 fields). In a subgroup of 20 patients in whom LUS was performed blindly by the two certified operators (C.O., R.F.), interobserver agreement was calculated. Each patient gave written informed consent to participate in the study; in case of patient's inability, the legally authorized delegate provided informed consent. The study protocol complied with the Declaration of Helsinki and was approved by the local Ethic Committee (n° protocol: CEAVNO-2020-17241).

Biochemical panel of inflammatory markers measurement and other measurements

Analysis for cytokines (IL-6) were performed in the laboratory of the Clinical Pathology Unit (University Hospital, Pisa) by a fully automated ELISA processing system (DSX DINEX Technologies) using commercial ELISA assays according to the manufacturer's instructions.

The following kits were used: Human IL-6 Instant ELISA Kit (Invitrogen, ThermoFisher Scientific).

C-reactive protein (CRP) was measured by high-sensitive assay on BN II nephelometer (Siemens Healthineers). Quantification of d-dimer was obtained by the assay Vidas® D-Dimer exclusion (bioMériuex) performed in the laboratory of Clinical Chemistry Unit (University Hospital, Pisa).

Hemogasanalysis was performed by GEM Premier 4000 Blood Gas Analizer (Werfen, Spain) and, gas exchange impairment was evaluated using arterial partial pressure of oxygen (PaO_2) to fraction of inspired oxygen (FiO^2) (P/F) [23]. According to NIH guidelines [3], patients were classified in two groups based on the CRP's cut off values used to start tocilizumab [24] patients with CRP values < 7.5 mg/dl were categorized as "controls", patients with a CRP value ≥ 7.5 mg/dl were categorized as "Hyperinflammated" patients.

Statistical analysis

Statistical analysis was performed by using SPSS 27.0 statistical software package (SPSS Inc., Chicago, IL) and GraphPad Prism 9. Continuous variables were presented as mean and standard deviation, ordinal variables as median and interquartile range (IQR), and categorical variables as percentage. Mann-Whitney and chi-square tests were used for multiple comparisons. Multivariate logistic regression analysis was performed to identify factors associated with "Hyperinflammation status" patients. Backward stepwise multivariate logistic regression was performed with the following continuous and categorical covariates: age, sex, heart failure, COPD, diabetes. Probability for removal of variables in the model was set at p = .10 or higher. Estimate odds ratios (ORs) with 95% confidence intervals (CIs) were obtained. Tests were performed considering a level of significance of 5%. Using Kendall's correlation, we assessed the possible correlation between the score obtained with chest ultrasound, the serum levels of C reactive protein (CRP), IL-6, ferritin and the values of the PaO_2/FiO_2 ratio. In order to estimate the threshold value most associated with cytokine storm, a ROC curve was calculated between the LUS score compared to CRP values ≥ 7.5 mg/dl used as a marker of hyperinflammatory response by COVID-19.

RESULTS

Our study examined a total of 65 geriatric patients [mean age 82.0 (SD = 6.9 years), 40% female] with a median Charlson Comorbidity Index (CCI) of 5 (IQR 3). Clinical and demographical characteristics of the cohort population are shown in Table I. Considering comorbidities, 36.9% had chronic heart failure; diabetes mellitus was present in 21.5 %, while 12.3% had COPD.

The indexed LUS score [mean (SD), 0.99 (0.61)] was inversely related to the PaO_2/FiO_2 ratio both at admission and nadir (respectively tau -0.29, and tau = -0.21, p < 0.01 for both), and directly related to the levels circulating of CRP, Ferritin and IL-6 (tau 0.35, 0.30 and 0.28 respectively, p < 0.001 for each, see Figure 1). In terms of in-hospital mortality, 14 patients of our cohort (21.5%) died (2 of them were transferred to ICU for non-invasive ventilation); we observed a statistically significant difference in LUS score values between deceased patients and controls [mean indexed LUS score 1.45 (SD = 0.62) *vs* 0.92 (SD = 0.58); respectively, p = 0.048]. Taking as reference the value of CRP at admission, according to NIH treatment guidelines [3], we identified patients with potential cytokine storm using the cut-off established by RECOVERY group trial [24] to start tocilizumab (≥ 7.5 mg/dl) and compared them to controls. Patients with hyperinflammation were less female than males (36% *vs* 64%) with a significant difference in PaO_2/FiO_2 ratio at nadir [mean PaO_2/FiO_2 nadir 161.3 (SD = 100.4) *vs* 234 (SD = 114); p 0.01] and at day 2 after admission [mean PaO_2/FiO_2 day2 202 (SD = 109.7) *vs* 350.9 (SD = 91.9), p 0.001] compared with the counterpart. No differences were found in terms of burden of comorbidities [median Charlson Comorbidity Index 5 (IQR = 3.25) *vs* 5 (IQR = 2), p 0.16]. Regarding LUS evaluation, we observed a significative difference of LUS score performed both on 12 [mean LUS score 12 fields 13.7 (SD = 7.5) *vs* 7.8 (SD = 5.2), p 0.04] and 16 fields [mean LUS score 16 fields 20.2 (SD = 7.5) *vs* 10.4 (SD = 8.7), p 0.01]. A high interobserver agreement between the LUS operators was found across the pre-defined subsample (k = 0.90). An indexed LUS score equal to or greater than

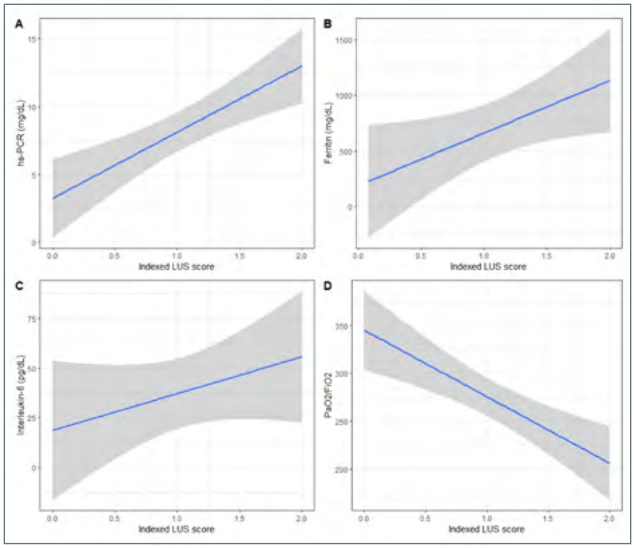


Figure 1. Correlation plots showing relationship between LUS score and hs-CRP (A), Ferritin (B), Interleukin-6 (C), PaO2/FiO2 (D).

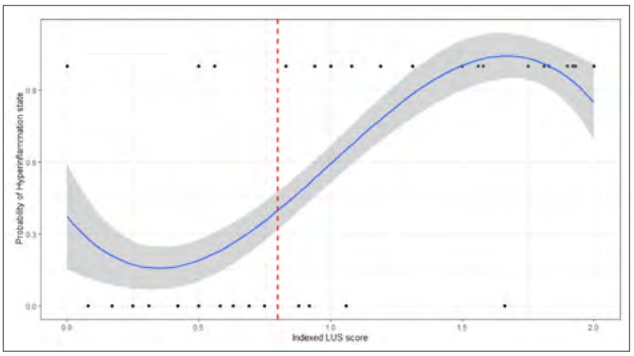


Figure 2. Non-linear smooth curve showing relationship between LUS-score and Hyper-inflammation state. The red dashed line represents LUS score cut-off for increasing probability of cytokine-storm.

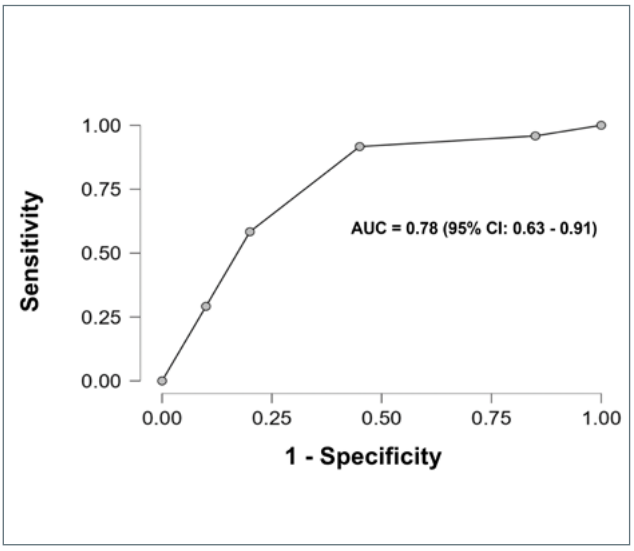


Figure 3. ROC curve of LUS score compared to "Hyperinflammation state".

0.8 (equivalent total score 9 on 12 fields and 12 on 16 fields) was highly predictive of the hyperinflammation state (86% Sensitivity, 68% Specificity), yielding a 0.78 AUROC ($p < 0.001$, see Figure 2 and Figure 3). Finally, indexed LUS score emerged as independent predictor of hyperinflammation and cytokine storm at multivariate analysis after adjusting for age, sex and heart failure, COPD and diabetes (LUS score adjusted OR = 5.90 95% CI: 1.59-21.66, p = 0.008).

DISCUSSION

Our study demonstrated that the higher the acute interstitial lung involvement evaluated by LUS, the higher the circulating levels of inflammatory markers such as CRP, IL-6, and ferritin in older patients with COVID-19 pneumonia. Notably, the LUS score at admission emerged as a strong predictor of cytokine storm and ARDS; furthermore, we determined a LUS score equal to or higher than 0.8 to be an accurate cut-off for hyper inflammation state.

These findings might be beneficial for the clinician's therapeutic decision-making and guide a timely immune-modulator therapy in the onset of severe COVID-19. From a pathophysiological point of view, the reason for the great sensitivity of LUS in the early detection of SARS-CoV-2 infection is due to that alterations in the lung parenchyma initially occur in the distal regions and then progress proximally [25]. These areas, which correspond to the "ground glass" opacities observed on CT scans [26], develop in the early stages of the disease and can be easily assessed with LUS.

Therefore, both LUS and CT have optimal accuracy in detecting early pulmonary involvement through apposite gravity scores that show a strong correlation with each other as reported in previous studies [27,28].

Some studies have shown how peculiar ultrasound patterns combined with the clinical characteristics of patients allow to exclude or not COVID-19 pneumonia with high accuracy [29,30]. In particular, a "high risk" ultrasound pattern emerged as a strong independent predictor of molecular swab positivity [31]. Accordingly, we confirmed an inverse correlation between PaO_2/FiO_2 ratio and LUS score, in agreement with Bosso et al. [32] and Rojatti et al. [33] reporting LUS score in SARS CoV-2 patients as negative related to disease severity and degree of hypoxemia, particularly in the presence of pleural effusion [18]. Regarding our cohort, we confirmed that LUS score correlates directly with the circulating levels of CRP, IL-6, and ferritin, in line with a recent report by Ji et al. found higher levels of CRP in the highest LUS score tertiles than patients in the low and moderate LUS score groups [26]. Consistent with our results, also Rubio et al. observed that patients with the highest LUS-score at baseline had a significantly lower PaO_2/FiO_2 ratio and higher concentration of LDH, CRP, and IL-6 [34]. For further analysis, we identified patients with a more likely risk of developing cytokine storm using the CRP cut-off value established from previous studies [24,35] and compared them with controls.

As expected, we found that patients showing a hyper-inflammatory state at admission had higher LUS score and lesser PaO_2/FiO_2 ratio than controls; meaning that the LUS score represents a good predictor of clinical deterioration at admission. In line with our findings, Lichter et al. study reported that higher LUS scores are strongly associated with disease worsening [36]. Similarly, in a geriatric cohort, Recinella et al. highlighted how LUS score showed a relevant

Table I. Characteristics of study population.

	Whole cohort (n = 65)	Patients with hyper-inflammation (n = 36)	Controls (n = 29)	P-value
Gender [F (%)]	26 (40)	13 (36)	13 (45)	0.47
Age [years, mean (SD)]	82 (6.9)	82.5 (7.2)	81.3 (6.5)	0.5
CCI [median (IQR)]	5 (3)	5 (3.25)	5 (2)	0.16
COPD (%)	8 (12.3)	5 (13.8)	3 (10.3)	0.66
Hypertension (%)	42 (64.6)	24 (66.7)	18 (62.1)	0.70
Heart failure (%)	24 (36.9)	12 (33.3)	12 (41.4)	0.50
Diabetes mellitus (%)	14 (21.5)	7 (19.4)	7 (24.1)	0.64
Stroke (%)	9 (13.8)	4 (11.1)	5 (17.2)	0.47
Dementia (%)	19 (29.2)	11 (30.6)	8 (27.6)	0.79
Chronic renal failure (%)	12 (18.5)	9 (25)	3 (10.3)	0.13
Fever on admission (%)	30 (46.2)	21 (58.3)	9 (31)	**0.02**
Cough (%)	14 (21.5)	11 (30.6)	3 (10.3)	**0.04**
Dyspnoea (%)	40 (61.5)	26 (72.2)	14 (48.3)	**0.04**
Fatigue (%)	14 (21.5)	6 (16.7)	8 (27.6)	0.28
Nausea or vomiting (%)	6 (9.2)	3 (8.3)	3 (10.3)	0.78
PaO_2/FiO_2 [on admission, mean (SD)]	277.7 (107)	255.2 (78)	308.1 (133)	0.06
PaO_2/FiO_2 [nadir, mean (SD)]	195 (112.2)	161.3 (100.4)	234 (114)	**0.01**
Lactate [on admission, mean (SD)]	1.42 (0.7)	1.5 (0.8)	1.2 (0.7)	0.12
WBC / mm^3 [on admission, mean (SD)]	7827 (3626)	8433 (2978)	7076 (4232)	**0.03**
Ferritin (mg/dL) [on admission, mean (SD)]	662.6 (727)	791.9 (788)	487 (615)	0.19
IL- 6 (pg/mL) [mean (SD)]	34 (51.1)	47.7 (63,4)	14.7 (8.2)	**0.04**
LUS scores 12 fields [mean (SD)]	10. 75 (7)	13.7 (7.5)	7.8 (5.2)	**0.04**
LUS scores 16 fields [mean (SD)]	16.5 (9.2)	20.2 (7.5)	10.4 (8.7)	**0.01**
LUS scores indexed [mean (SD)]	0.9 (0.6)	1.25 (0.5)	0.7 (0.5)	**0.002**

COPD: Chronic Obstructive Pulmonary Disease; LUS: Lung Ultrasound Score; PaO_2/FIO_2 ratio, partial pressure arterial oxygen/fraction of inspired oxygen ratio; WBC: White Blood Cell; CCI: Charlson Comorbidity Index.

prognostic role in the stratification of in-hospital mortality [37]. Furthermore, in agreement with Tana et al. [28] that firstly evaluated the prognostic role of LUS in stratifying mortality risk, we reported higher median LUS score values at admission in deceased patients compared to controls.

Accordingly, Rubio et al. and Li et al. found that LUS score was significantly negatively correlated with PaO_2/FiO_2 ratio and positively correlated with inflammatory markers and scores of clinical deterioration, such as APACHE II, in most severely affected patients [25,34]. Recently, three studies already used the LUS score as a risk stratification tool for COVID-19 patients [26,34,36]. These papers proposed their cut-off value of LUS score to determine the severity of COVID-19 [25,36-40]. The cut-off values for predicting severity and consequent reduction of survival rate were variable and ranged from 13 [39], 16 [38], 17 [37], 18 [36] up to 22.5 [25], respectively.

In our cohort, an indexed LUS score greater than 0.8 was found to be the most predictive cut-off for cytokine storm, indicating a lower value than that reported in the aforementioned papers, carried out in younger cohorts. This discrepancy may be due to the impaired and ineffective inflammatory response observed in oldest patients with cytokine storm, in agreement with a recent paper describing a decreased number of ground-glass opacities detected by CT, in oldest patients with COVID-19 compared to younger peers [41]. This finding suggests that LUS scores usually associated with mild-moderate COVID-19 pneumonia may be instead predictive of cytokine storm in older patients, thus indicating the timing for prompt therapeutic intervention. The findings in this report are subject to at least three limitations. Firstly, with small sample size, caution must be applied, as the findings might not be transferable to different cohorts of older patients with COVID-19. Secondly, the single-center design study might have reduced the generalizability of our results; notwithstanding, the monocentric investigation allowed an accurate and standardized data collection. Moreover, it is important to bear in mind that ultrasonography is a highly operator-dependent imaging modality with a number of operator-related variables that can impair or enhance image quality, leading to misdiagnosis.

Therefore, further prospective multicentre studies are warranted to confirm the predictive role of LUS in older patients hospitalized for COVID-19 pneumonia.

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Conflict of interest statement
The Authors declare no conflict of interest.

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Author contributions
RF, CO: performed the study concept and design; VM, TM, DG, SR, UB: contributed to the acquisition of data; CO, RF: performed the analysis and interpretation of data; RF, CO, AP, GC, AF: were responsible for the drafting of the manuscript; FM, CO: provided the critical revision of the manuscript for important intellectual content. All Authors have read and agreed to the published version of the manuscript.

Ethical consideration
This study was approved by the Institutional Ethics Committee (Comitato Etico Regionale per la Sperimentazione Clinica della Regione Toscana, Area Nord Ovest) (protocol number CEAVNO-2020-17241).
The research was conducted ethically, with all study procedures being performed in accordance with the requirements of the World Medical Association's Declaration of Helsinki.
Written informed consent was obtained from each participant/patient for study participation and data publication.

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