Surfactant-derived protein type B: a new biomarker linked to respiratory failure and lung damage in mild to moderate SARS-CoV-2 pneumonia

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Immature surfactant protein B (SP-B) emerges as a potential biomarker for assessing severity and prognosis of COVID-19 pneumonia. SP-B correlates with lung involvement and oxygen support requirement, suggesting its utility in disease monitoring. https://bit.ly/3LcdTbp

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Abstract

Background The COVID-19 pandemic has led to significant concern due to its impact on human health, particularly through pneumonia-induced lung damage. Surfactant proteins A and D (SP-A and SP-D) are implicated in COVID-19 lung damage, but the role of surfactant protein B (SP-B) remains unclear.

Methods We conducted a single-centre, prospective observational study involving 73 hospitalised COVID-19 pneumonia patients. SP-B levels were measured within 48 h of admission, alongside SP-A and SP-D in a subset. Clinical data were collected, and follow-up visits were conducted after 6 months.

Results At hospitalisation, circulating immature SP-B levels measured in 73 patients (median 26.31 arbitrary units (AU) (interquartile range 14.27–41.31)) correlated significantly with lung involvement ($r=0.447$, $p<0.001$) and oxygen support requirement ($p=0.005$). SP-B levels did not predict mechanical ventilation or intensive care unit admission. SP-B decreased significantly ($p<0.001$) from 25.53 AU (14.36–41.46) at the acute hospitalisation to 12.73 AU (9.12–20.23) at the 6-month follow-up, whereas SP-A and SP-D did not change significantly. Immature SP-B (but not SP-A and SP-D) was confirmed to be significantly associated with the need for oxygen support (n=26, 58%) during the hospitalisation ($p<0.05$).

Conclusion Immature SP-B emerges as a potential biomarker for COVID-19 pneumonia severity and prognosis. Its dynamic changes suggest utility in monitoring disease progression and long-term outcomes, despite limitations in predicting hard end-points. Larger studies are needed to validate these findings and understand the underlying mechanisms of surfactant protein dysregulation in COVID-19 pathogenesis.

Introduction

The COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has sparked widespread concern globally due to its severe implications for human health. One of the most frequent and occasionally severe manifestation of the disease is pneumonia, which can manifest in mild or severe forms, often leading to significant lung damage. In this context, the pulmonary surfactant proteins A and D (SP-A and SP-D) have emerged as a particularly intriguing biomarkers, as they are closely related to lung damage [1, 2] and may play a critical role in the lung's response to COVID-19 infection [3–6].

However, despite the well-known relevance of surfactant protein B (SP-B) in the context of lung damage from different aetiologies, including heart failure (HF) [7–10], its levels both during acute COVID-19 infection and in the post-acute phase have not yet been analysed. Therefore, its variation within the course of infection and its relationship with the degree of lung damage remain to be clarified. Of note, SP-B is

produced only in alveolar cells. In contrast, SP-A and SP-D have several extra-thoracic production sites and have been previously associated with immunity and pathology condition of the lung [11–13], including COVID-19 infection. Indeed, both SP-A and SP-D have been reported to increase during COVID-19 [3–5].

The purpose of this study was to investigate SP-B levels during acute COVID-19 infection and at a medium-term follow-up, particularly examining the relationship of SP-B with lung damage and respiratory failure. Furthermore, we have also explored the behaviour of SP-A and SP-D in this context, to gain a more comprehensive understanding of the role of pulmonary surfactant proteins as potential biomarkers of lung damage and healing during COVID-19.

Methods

This was a single-centre, observational, prospective study. We enrolled patients hospitalised in our hospital for pneumonia with a positive swab for SARS-CoV-2 between March 2020 and April 2021. Patients were treated according to the routine clinical practice at the moment of the hospitalisation. Within 48 h from admission, we collected blood samples to measure SP-B (both mature and immature form). Additionally, we also measured SP-A and SP-D in a subpopulation. SP-B (immature form) was measured by Western blotting on plasma samples, as previously described [14]. Plasma levels of mature SP-B, SP-D and SP-A were determined using commercially available ELISA kits. The intra-assay and inter-assay coefficients of variation were <10% and <12% for the SP-B ELISA (Cloud-Clone Corp., Houston, TX, USA), <5.2% and <3%, for the SP-D ELISA (BioVendor, Heidelberg, Germany) and <8% and <10% for the SP-A ELISA (Biomatik, Kitchener, Canada).

Clinical data, encompassing demographic details, medical background, physical manifestations, laboratory findings and radiographical images, were collected from the medical files of individuals. Standard blood assessment results were collected among those conducted for clinical reasons. Chest computed tomography (CT) scans were performed to hospitalised individuals, as prescribed by the attending physician. Specifically, CT examinations were performed using a 256-slice high-resolution CT scanner (Revolution CT; GE Healthcare, Milwaukee, WI, USA). No contrast media were administered to the patients. The percentage of extension of lung parenchyma affected by COVID-19 pneumonia was processed by a dedicated workstation (ADW4.6, GE Healthcare) using a specific reconstruction software (Thoracic-V-Car software; GE Healthcare). This quantitative approach enables an automated assessment of the pulmonary infection, depicting infection areas as high attenuation areas (HAAs) in respect of a defined threshold value ranging from 650 HU to 3071 HU. The amount of infected lung defined as the percentage of lung parenchyma above the predefined vendor-specific threshold of 650 HU (HAA%, HAA/total lung volume) was automatically calculated by the dedicated software for both lungs [15, 16].

After 6 months, patients underwent a follow-up visit and repeated blood samples. Clinical history and results of tests were collected according to the local regulation after the patient signed the appropriate informed consent. The study was approved by the Ethics Committee of Centro Cardiologico Monzino and registered as R1174/20 CCM 1237.

Statistical analysis

Statistical analysis was performed using SPSS 25.0 software (SPSS Inc., Chicago, IL, USA) and SAS version 9.4 (SAS Institute, Cary, NC, USA). Continuous variables were expressed as mean±SD or median and (interquartile range) as appropriate, while discrete variables were expressed as absolute numbers and percentages. Comparisons between basal variables and follow-up were performed using a Wilcoxon signed-rank test. A Spearman correlation was used for non-normally distributed variables, while logistic regression was used to evaluate the association between SP-B and outcomes. A p-value $\leqslant 0.05$ was considered as statistically significant.

Results

From March 2020 to April 2021, we enrolled 73 patients (mean age 72 (interquartile range 63–79) years; 62% males) admitted to Centro Cardiologico Monzino, IRCCS (Italy) due to COVID-19 pneumonia. In brief, 59% were hypertensive, 30% were active or previous smokers and 65% had a pre-existing cardiopulmonary disease, including 19% with HF and 10% with COPD. All patients were hospitalised in a clinical ward dedicated to COVID-19 patients. The median hospitalisation length was 11.0 (7.0–18.5) days; 15% of patients during hospitalisation due to clinical worsening were transferred to the intensive care unit (ICU) but only one patient needed invasive mechanical ventilation. 44 patients needed oxygen support. No deaths occurred during hospitalisation but five patients died in the following 6 months after the discharge for reasons unrelated to COVID-19.

We detected 26.31 arbitrary units (AU) (14.27–41.31) of circulating immature SP-B, whereas the mature form was undetectable in blood. Immature SP-B was significantly correlated with lung involvement at computed tomography (CT) analysis (r=0.447; p<0.001). Additionally, immature SP-B was significantly higher in those patients who required some sort of oxygen support, including nasal cannula, Venturi mask, continuous positive airway pressure (CPAP) or invasive mechanical ventilation (n=44, 60%) during hospitalisation (31.58 (17.45–48.39) *versus* 16.19 (10.81–19.13) p=0.003), with a significant association at logistic regression (p=0.005). Conversely, immature SP-B was not associated with the occurrence of hard end-points such as invasive mechanical ventilation, use of CPAP or ICU admission, nor it was correlated with hospitalisation duration. Finally, no association was found between immature SP-B and history of cardiopulmonary disease.

45 patients (62%) were re-evaluated at 6 months after hospital discharge at a follow-up visit. The remaining 28 patients did not attend the scheduled follow-up appointment for various reasons (figure 1). The main characteristics of this population with acute and medium-term evaluation are reported in table 1, presenting the data collected at enrolment (hospitalisation). These patients had an SP-B (mature and immature form), SP-A and SP-D assay twice, during hospitalisation (within 48 h of hospital admission) and at the follow-up visit. Figure 2 shows that immature SP-B significantly reduced at 6 months, whereas SP-A and SP-D did not change. Notably, also in this subgroup, immature SP-B (but not SP-A and SP-D) was confirmed to be significantly associated with the need for oxygen support (n=26, 58%) during the hospitalisation (p<0.05) with a lower value of immature SP-B of 20.60 (13.41–29.46) *versus* 30.67 (16.44–48.39); p<0.05) in patients who did not need oxygen supplementation (figure 3).

Excluding patients with known HF (n=8), the results confirmed a significant decrease of immature SP-B at the 6-month follow-up (24.35 (14.24–44.27) *versus* 12.54 (8.28–20.0), p<0.001) and a persistent significant correlation of in-hospital immature SP-B value with oxygen need (p=0.038 at logistic regression).

All the tested haematological variables reported in table 1 were evaluated against surfactant proteins but there were no significant correlations.

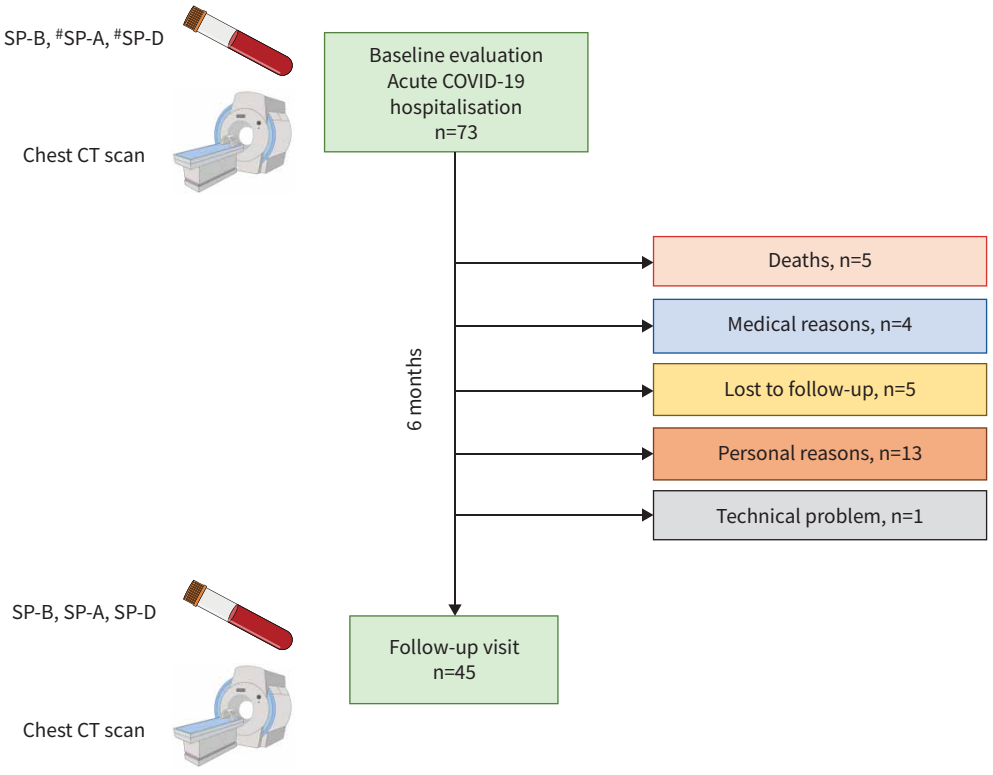

FIGURE 1 Flow chart presents the two main steps of the study with the reasons why some patients did not attend the follow-up visit. SP: surfactant protein; CT: computed tomography. #: n=45.

TABLE 1 Basal (hospitalisation) characteristics of the 45 patients with acute and 6-month follow-up

	n (%)	Median (IQR)
Age, years	45	71.50 (64.50–78.00)
Sex, male	29 (64.4)	
Medical history		
Previous history of CP disease	28 (62.2)	
Active smokers	4 (8.9)	
Previous smokers	10 (22.2)	
Hypertension	29 (64.4)	
Diabetes	14 (31.1)	
Dyslipidaemia	20 (44.4)	
Atrial fibrillation	17 (37.8)	
Arrythmia	4 (8.9)	
Ischaemic cardiomyopathy	15 (33.3)	
Previous myocardial infarction	12 (26.7)	
Heart failure	8 (17.8)	
Valvulopathy	6 (13.3)	
Stroke	2 (4.4)	
Previous CV hospitalisations	17 (37.8)	
COPD	4 (8.9)	
Hospitalisation data		
Hospitalisation length, days	45	10.00 (5.75–16.00)
Intensive care	5 (11.1)	
Deaths during hospitalisation	0 (0)	
Need of any oxygen therapy	26 (58)	
Highest level of O_2 support		
O_2 low flow	13 (28.9)	
CPAP	12 (26.7)	
Venturi	0 (0)	
Intubation	1 (2.2)	
CT lung involvement, %	43	11.23 (7.51–18.43)
hsTnI at admission, ng·L^{-1}	32	20.80 (6.25–64.54)
hsTnI highest, ng·L^{-1}	28	65.00 (15.01–1694.32)
BNP, pg·mL^{-1}	42	243 (74.00–379.00)
CRP at admission, mg·L^{-1}	45	23.30 (9.05–52.85)
CRP highest, mg·L^{-1}	45	53.95 (25.20–139.45)
Leukocytes, 10^3·µL^{-1}	45	7.40 (5.85–9.53)
Leukocytes highest, 10^3·µL^{-1}	45	10.30 (8.28–13.93)
ST2, ng·mL^{-1}	25	70.80 (38.55–100.55)
D-dimer peak, ng·mL^{-1}	39	821.21 (575.45–1411.18)

Data are presented as n (%) or median (interquartile range (IQR)). CP: cardiopulmonary; CV: cardiovascular; CPAP: continuous positive airway pressure; CT: computed tomography; hsTnI: high sensitive troponin-I; BNP: B-type natriuretic peptide; CRP: C-reactive protein; ST2: soluble interleukin 1 receptor-like 1.

Discussion

Our investigation, conducted during the most severe phase of the COVID-19 pandemic [17], revealed for the first time, a significant association between circulating immature SP-B levels and the extent of lung involvement assessed by CT analysis [18–20]. Furthermore, higher immature SP-B levels were observed in patients requiring oxygen support, suggesting a potential role of immature SP-B as a biomarker for respiratory distress in COVID-19 pneumonia.

The population we studied was characterised by acute COVID-19 infection associated with pneumonia. Of note, patients directly admitted to the ICU were not considered in the present analysis, which focuses on patients with only moderate respiratory distress severity, who need hospitalisation but were not considered in need of intensive care at presentation. Indeed, the clinical profile of our patients revealed a high prevalence of comorbidities, with hypertension being the most common, followed by a history of smoking and pre-existing cardiopulmonary diseases such as HF and COPD (table 1). These findings may reflect the peculiar nature of the hospital where the trial was conducted (a centre specialised in cardiovascular care), but also align with previous studies [21, 22] highlighting the association between COVID-19 severity and underlying health conditions.

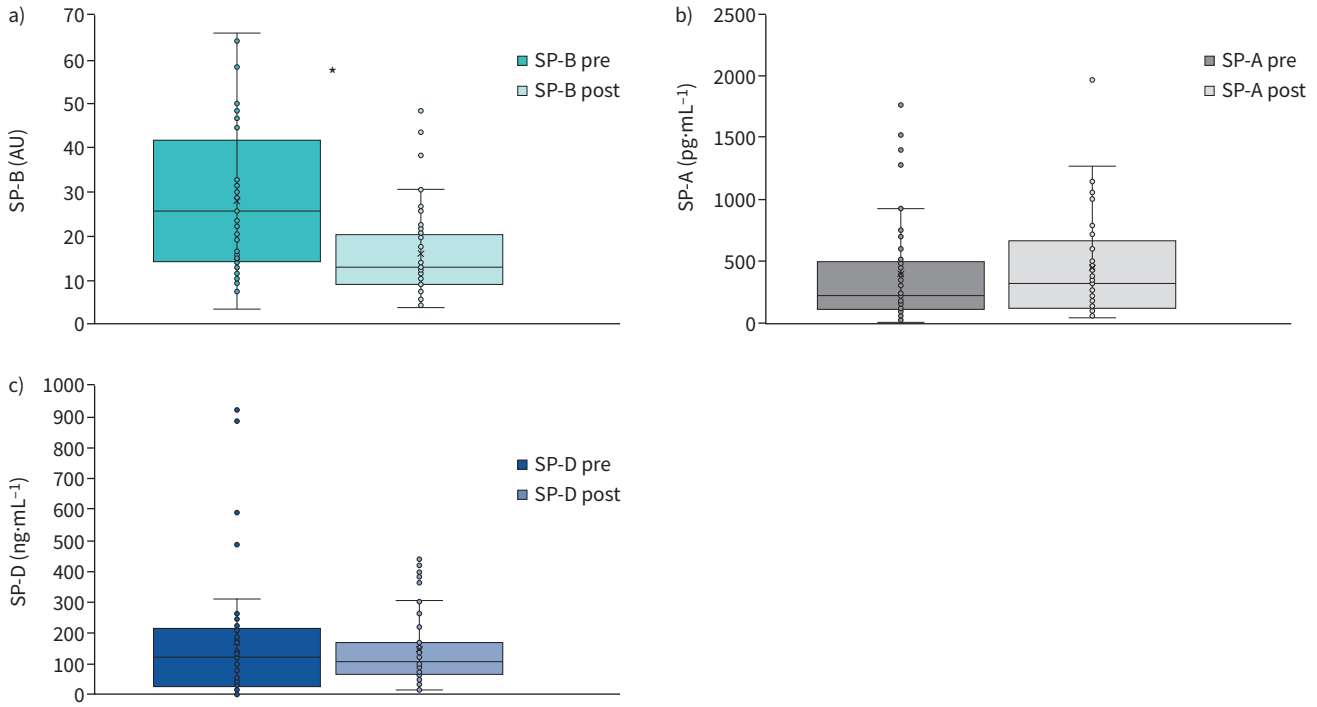

FIGURE 2 Evaluation of a) surfactant binding protein (SP)-B (immature form), b) SP-A and c) SP-D at enrolment (hospitalisation due to COVID-19) and at follow-up (6 months). AU: arbitrary units. *: p<0.01.

The role of SP-B, both mature and immature forms, as markers of alveolar cells distress is already known in different clinical contexts such as HF and respiratory diseases [7–9, 14]. As a protein contained only within the alveolar cells, the release of immature SP-B in the blood signifies established alveolar damage, regardless of the cause (HF, COPD, acute respiratory distress syndrome, and physical stressors such as diving and smoke) [14, 23–27]. Indeed, in chronic HF a strong correlation was previously reported between circulating immature SP-B and alveolar–capillary membrane gas diffusion [14]. In contrast, to the best of our knowledge no data exist on SP-B behaviour, both in the mature and immature form, in cases of

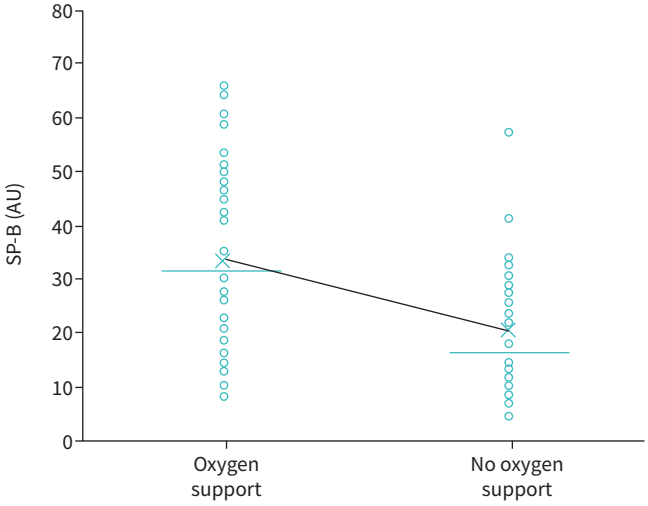

FIGURE 3 Association between immature surfactant binding protein (SP)-B and oxygen support during hospitalisation. Dots represent individual measurements, crosses represent the median value and lines represent the means. AU: arbitrary units.

acute non-COVID-19 pneumonia in adults. Of note, our results were confirmed when we excluded patients with HF, confirming the role of SP-B as a marker of alveolar damage, which can occur from both sides of the alveolar–capillary membrane: the circulatory side (HF) and the pulmonary side (COVID-19). While altogether these findings make SP-B less specific from a purely diagnostic point of view compared with other biomarkers, its importance in assessing lung involvement in several diseases remains crucial.

COVID-19 has proven to be a systemic illness, affecting numerous organs and tissues [28–32]. Yet, also considering the virus's point of entry (the respiratory system), the lung has consistently emerged as the primary target of the disease, with the hallmark of interstitial pneumonia described since the earliest documented cases in China [33]. In recent years, many prognostic biomarkers have been associated with COVID-19 severity and prognosis [29, 30, 34–39]; however, most of these are not lung-specific. Given its peculiar biology, SP-B can play a crucial role in this context, identifying significant lung involvement from the early stages of the disease with possible clinical and therapeutic repercussions. However, despite the association between immature SP-B levels and lung damage on a CT scan and between immature SP-B levels and oxygen requirement during hospitalisation, we did not observe significant correlations with hard end-points such as intubation, need of CPAP and/or ICU admission. This could be related to the small sample considered and due to a relevant number of nonsevere COVID-19 cases, as confirmed by the absence of in-hospital deaths. Additionally, immature SP-B levels were not predictive of hospitalisation length. The demographic characteristics of our cohort, summarised in table 1, depict a predominantly older male population with a median age of 72 years, consistent with the demographics of COVID-19 patients reported in other studies [39, 40].

Interestingly, while immature SP-B levels exhibited a significant reduction at the 6-month follow-up visit, SP-A and SP-D levels remained unchanged. This finding underscores the unique dynamics of immature SP-B in the context of COVID-19 recovery. Indeed, differently from previous studies we did not find a correlation between SP-A/SP-D values and clinical condition at hospitalisation or clinical improvement with time. This is likely related to the low prevalence in our population of severe COVID-19 pneumonias in the acute phase, reinforcing the clinical role of SP-B (immature form), which is associated with clinical condition at hospitalisation and after recovery. Altogether these findings highlight the potential utility of immature SP-B as a prognostic marker for assessing acute lung damage as well as long-term respiratory outcomes, even in subjects with moderate lung disfunction. These findings, however, deserve to be validated in larger studies as well as in patients with more severe COVID-19 pneumonia.

Limitations

This is a monocentric study with a relatively small sample size; thus, the results would need validation in a larger cohort as well as in cohorts with patients with more severe COVID-19 pneumonia lung involvement. This limitation is relevant for several possible subanalyses.

Moreover, the patients were affected by the early forms of the SARS-CoV-2 virus, which has since undergone significant mutations (patients were enrolled during the so-called "first and second waves" in Italy). It is crucial to determine whether a more attenuated virus can elicit the same or different responses. Similarly, we lack information on surfactant proteins response in vaccinated individuals as we do not know whether the values of the various surfactant proteins obtained at enrolment were the highest. Indeed, we had only two measurements: at the time of hospitalisation and 6 months after. Therefore, the time-related changes of surfactant proteins are unknown. Finally, a limited number of patients returned for follow-up visits, resulting in insufficient data for robust correlations with long-term clinical outcomes.

Conclusions

In conclusion, our study provides valuable insights into the clinical characteristics and biomarker profiles of COVID-19 pneumonia patients, highlighting the potential utility of immature SP-B as both a marker of pneumonia severity and a prognostic indicator for respiratory outcomes. Future research should focus on validating these findings in larger cohorts and exploring the underlying mechanisms driving surfactant protein dysregulation in COVID-19 pathogenesis.

Provenance: Submitted article, peer reviewed.

Data availability: Raw data will be available upon request at https://zenodo.org/badge/DOI/10.5281/zenodo.13885724.svg.

Ethics statement: The study was approved by the Ethics Committee of Centro Cardiologico Monzino and registered as R1174/20 CCM 1237. Each subject provided written consent to the study.

Author contributions: P. Agostoni, M. Mapelli, E. Salvioni, I. Mattavelli and S. Harari wrote the main manuscript text. C. Banfi, A. Greco and S. Ghilardi performed the surfactant binding protein analysis. M.L. Biondi performed all other laboratory assessments. E. Salvioni performed statistical analysis. S. Rovai collected data. E. Mancini analysed thoracic computer tomography data. All authors reviewed and approved the manuscript.

Conflict of interests: None declared.

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