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ORIGINAL

# Type III procollagen is a reliable marker of ARDS-associated lung fibroproliferation

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**Take-home message:** The determination of Procollagen III on broncho-alveolar lavage done at day 7 in persistent ARDS is able to identify patients with fibroproliferation.

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**Abstract** *Purpose:* A specific biomarker of post-ARDS fibroproliferation could be useful in the identification of patients who could benefit from therapies aiming to modulate fibroproliferation such as corticosteroids.The aim of this prospective study was to determine the best threshold of the N-terminal-peptidetype III procollagen (NT-PCP-III) in non-resolving ARDS to validate this threshold according to the outcome. *Methods:* Concerning the best threshold of NT-PCP-III, all consecutive patients with a non-resolving ARDS were included if all the following criteria were fulfilled: moderate to severe ARDS lasting for at least 5 days, lung biopsy performed, serum and alveolar NT-PCP-III obtained within 1 week prior to biopsy, and no documented infection contra-indicating the corticosteroids. In the validation cohort part of the study, patients were included at day 7 if they presented a persistent moderate to severe ARDS.
*Results:* Nineteen of 32 patients had fibroproliferatio nonbiopsy. Serum and alveolar NT-PCP-III were higher in patients with fibroproliferation. Using a threshold of 9 μg/L, alveolar NT-PCP-III had the highest accuracy for diagnosing fibroproliferation (sensitivity = 89.5 % and

specificity = 92.3 %). Regarding the 51 patients included in the validation cohort, the mortality rate at day 60 was increased in patients presenting an alveolar NT-PCP-III level higher than 9 μg/L (69 vs. 17 %, $p < 0.001$). The mean alveolar level of NT-PCP-III on day 7 was 8.1-fold higher in nonsurvivors ($p = 0.03$). *Conclusions:* The determination of NT-PCP-III on BAL done at day 7 in persistent ARDS is able to identify patients with fibroproliferation who could be included in a trial of corticosteroids or any other treatment that might help resolve lung fibroproliferation.

**Keywords** Biopsy · Accuracy · Sensitivity · Comparative

## Introduction

Acute respiratory distress syndrome (ARDS) is associated with a high mortality rate of 30–60 % [1–4]. Pulmonary fibroproliferation is a possible evolution of acute respiratory distress syndrome (ARDS). Indeed, results from histopathological studies show that an early inflammatory phase is followed by a fibroproliferative repair phase, with cell proliferation and deposition of matrix proteins leading to the resolution of ARDS or to irreversible lung fibrosis and death [2]. It has been shown that this ARDS-associated lung fibrosis is associated with a poor outcome [5–7]. The diagnosis of ARDS-associated pulmonary fibroproliferation is difficult to establish. The gold standard remains a lung biopsy and a histological examination. The use of therapies able to alter fibroproliferation such as corticosteroids in this issue has been suggested but remains controversial [8–10]. It has been shown that approximately 50 % of the ARDS patients who had an open-lung biopsy (and in whom corticosteroids were potentially indicated) did not exhibit any sign of fibroproliferation by histological analysis [11]. The use of a highly specific biomarker of pulmonary fibroproliferation could therefore be useful in the early identification of a population that could benefit from therapies able to alter fibroproliferation.

Pulmonary fibroblasts produce procollagen, which is a precursor of collagen. The NT part of procollagen III, which results from the enzymatic cleavage of procollagen by specific proteases in the extracellular space, is used as a marker of collagen synthesis [12, 13]. Serial assessments of the N-terminal peptide for type III procollagen (NT-PCP-III) have been carried out in broncho-alveolar lavage (BAL) and blood from ARDS patients and are well correlated with the outcome [14, 15]. An early rise, in the first 24 h of ARDS, of the alveolar concentration of type III procollagen is associated with increased mortality [14, 15]. Type III procollagen was therefore validated as a severity marker of ARDS but not as a biomarker of fibroproliferation. A relationship has never been established between this increase in NT-PCP-III and the presence of lung fibroproliferation, except in a small series of 8 ARDS patients [16]. Interestingly, Steinberg et al. [10] more recently showed, in a secondary objective of the LASARUS study, that ARDS mortality was lower in patients who received corticosteroids while presenting a high level of type III procollagen on admission. It could therefore be of utmost importance to guide corticosteroids treatment using type III procollagen if this biomarker is associated with the presence of fibroproliferation and not simply related to the ARDS severity.

However, to our knowledge, NT-PCP-IIIin BAL fluid/serum has never been compared to the lung histological gold standard in ARDS patients ventilated according to a lung-protective strategy and presenting a non-resolving ARDS. Thus, the aim of this prospective study was to determine the best threshold of both the serum and the alveolar concentrations of the NT-PCP-III in non-resolving ARDS patients, using lung histological examination as the reference method, and to validate this threshold according to the outcome in a prospective cohort of consecutive patients presenting a persistent ARDS.

## Methods

This study was conducted from January 2008 to June 2012.

### Patients

#### *Determination of type III procollagen threshold on BAL/serum*

This prospective observational part of the study was conducted in the medical ICU of a teaching hospital.

#### *Validation cohort*

This prospective observational cohort part of the study was performed in the medical ICU of a teaching hospital, in a medico-surgical ICU of a teaching hospital, and in the medico-surgical ICU of a non-teaching hospital. The investigation was approved by the local ethics committee. Written informed consent was obtained from patients and/or next of kin prior to enrollment.

## Inclusion criteria

### *Determination of type III procollagen threshold on BAL/serum*

All consecutive patients presenting with a non-resolving ARDS were included if all the following criteria were fulfilled: moderate to severe ARDS lasting for at least 5 days with a $PaO_2/FIO_2$ ratio (P/F ratio) <200 mmHg and a PEEP of at least 5 $cmH_2O$ [17, 18], lung biopsy performed, and serum and alveolar type III procollagen obtained within 1 week prior to lung biopsy.

### *Validation cohort*

Patients over 18 years of age were included at day 7 if they presented a persistent ARDS and were still under invasive mechanical ventilation. Only moderate to severe ARDS patients with a P/F ratio <200 mmHg at a positive end-expiratory pressure (PEEP) ≥5 $cmH_2O$ were included [17, 18].

## Non-inclusion criteria

If a patient presented at least one of the following diagnoses, he/she was not included: chronic interstitial or fibrotic lung disease, acute or chronic liver disease, corticosteroid (>200 mg/day of hydrocortisone or equivalent at any moment during the month preceding inclusion), immunosuppressive therapy within the last 30 days, presence of an advanced directive to withhold life-sustaining treatment, and persistent (more than 4 h) $PaO_2/FIO_2$ <70 mmHg despite maximal treatment (safety criteria for BAL).

## Usual patient care

Volume-assist-control ventilation was used at the early phase of ARDS with a tidal volume of 6–8 mL/kg of predicted body weight. The plateau pressure was maintained to not exceed 30–32 $cmH_2O$. The oxygenation goal was a pulse-oxymetry arterial saturation ($SpO_2$) of 88–95 % or a $PaO_2$ of 55–80 mm Hg. To achieve this goal, the $FIO_2$ and PEEP were adjusted as in the ARMA trial [1]. Rescue treatments were used if necessary (prone positioning, inhaled nitric oxide, almitrine bismesilate, oscillatory ventilation, or extra-corporeal membrane oxygenation).

## Blood and bronchoalveolar lavage fluid collection

BAL fluid was obtained from the most infiltrated lung area on chest X-ray. Sterile saline was instilled in one aliquot of 50 mL. If the recovered lavage fluid was <10 mL, a second 50-mL of sterile saline aliquot was administered.

## Using the N-terminal peptide to measure type III procollagen

Five ml of the BAL was centrifuged at 3,500 rpm for 10 min. The supernatant was stored at −70 °C and later batch-assayed. Serum and alveolar NT-PCP-III were measured using a radioimmunological method (UniQ-procollagen III Radioimmunoassay; Orion Diagnostica, Espoo, Finland). For serum NT-PCP-III, a normal value for healthy people is 3.7 μg/L (2.5th and 97.5th percentiles, from 2.3 to 6.4 μg/L) using this technique. The sensitivity threshold is 0.3 μg/L. The intra-test variability is 3, 7 and 4.1 % for NT-PCP-III levels of 2.8, 6.6 and 11.9 μg/L, respectively. The inter-test variability is 6.5, 4.5 and 7.2 % for NT-PCP-III concentrations of 2.7, 6.8 and 7.2 μg/L, respectively. These characteristics given by the manufacturer were validated by our laboratory.

## Open-lung biopsy

An open-lung biopsy (OLB) was performed in the first part of the study related to determination of type III procollagen threshold. The OLB technique has been previously described [11]. Briefly, an OLB was performed by an anterior thoracotomy, and preferentially in the right middle lobe. When the left lung was the most injured (chest X-ray and/or CT scan), OLB was performed in the lingula. The OLB was generally performed at the bedside. Two chest drains were inserted at the end of the procedure. Parts of the biopsy were sent for microbiological analysis and for histological assessment. As usually done in our ICU, OLB was performed only when the microbiological results of BAL and blood samples were available.

### *Histological assessment*

The lung sample was treated with 10 % formaldehyde at room temperature for a 24-h period. After dehydration, the lung biopsy was embedded in paraffin. Ten 4-μm slices were examined after hematoxylin-eosin staining. The pathologists were blinded to all the information regarding lung mechanical properties. The biopsies were read by two independent, trained and experienced pathologists (V.S., M.J.P.). A collegial analysis was done in case of disagreement. A total of 30–35 spots were analyzed (magnification ×100) to classify the biopsy according to the following previously described scale: stage 0 (no), normal lung structure; stage 1 (mild), mild interstitial fibroproliferation; stage 2 (moderate), moderate interstitial

fibroproliferation; and stage 3 (extensive), extensive fibroproliferation with lung architecture distortion and alveolar obliteration by dense fibrous material [7].

### Statistical analysis

The distribution was evaluated using the Kolmogorov–Smirnov test. According to the distribution, quantitative variables were expressed as the median and interquartile range (IQR) (25th percentile and 75th percentile) or mean (±standard deviation) and the comparisons between groups were performed using the Mann–Whitney *U* test or Student's *t* test. Qualitative variables were compared using the Fisher exact test. Because, in the validation cohort, the LIS was significantly higher in patients with increased alveolar NT-PCP-III as well as in nonsurvivors, we performed a logistic regression analysis (entry model) to identify if alveolar NT-PCP-III was independently associated with ICU death. We included in the Cox model the LIS and the NT-PCP-III alveolar level. The diagnostic accuracy of NT-PCP-III was reported in accordance with the guidelines of the standards for the reporting of diagnostic accuracy studies (STARD) [19]. Receiver operating characteristic (ROC) curves were constructed to evaluate the diagnostic value of NT-PCP-III for diagnosing lung fibroproliferation. Areas under the ROC curves (AUC) were determined. Comparisons between AUC were performed using the Hanley method with MedCalc Software v.12.3.0 (Mariakerke, Belgium) [20]. According to the ROC curve, the value of NT-PCP-III that exhibited the best accuracy was chosen as the threshold. The sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV) and likelihood ratio (LR) were determined, using OpenEpi v.2.3.1 for serum and alveolar NT-PCP-III [21]. The confidence intervals for the Se, Sp, PPV and NPV were determined using the Wilson score method [22]. The confidence intervals for the positive and negative LRs were calculated using the Simel method [23]. The correlation between fibroproliferation intensity and NT-PCP-III was performed using a Spearman test. A $p$ value $<0.05$ was considered to be significant. If not mentioned, statistical analysis was conducted using SPSS v.20.0 (IBM, New York, USA).

## Results

### Determination of type III procollagen threshold on BAL/serum

#### *Patient characteristics*

Forty-nine patients presented with persistent ARDS and underwent a lung biopsy during the study period. Seventeen of them were not included (lung cancer, 10; cirrhosis, 1; interstitial pneumonia, 4; receiving corticosteroids, 2). The remaining 32 patients were therefore included. The

**Table 1** General characteristics of the 32 patients with persistent ARDS who underwent a lung biopsy

| | All ($n = 32$) | Fibroproliferation ($n = 19$) | No fibroproliferation ($n = 13$) | $p$ value |
|---|---|---|---|---|
| Age (years)[a] | 64 (55–71) | 61 (55–75) | 65 (55–69) | 0.80 |
| SAPS II score on admission[a] | 44 (37–51) | 43 (35–49) | 44 (38–57) | 0.55 |
| Main cause of ARDS [*n* (%)] | | | | 0.12 |
| Aspiration | 6 (19) | 3 (16) | 3 (23) | |
| Infectious pneumonia | 21 (66) | 15 (79) | 6 (46) | |
| Extra-pulmonary sepsis | 3 (9) | 0 (0) | 3 (23) | |
| Trauma | 2 (6) | 1 (5) | 1 (8) | |
| SOFA the day of biopsy[a] | 9 (6–14) | 11.5 (7.5–14.5) | 7 (6–14) | 0.19 |
| Duration of ARDS prior biopsy, days[a] | 10 (7–12) | 10 (6–12) | 10 (8–13) | 0.60 |
| Tidal volume, mL/kg PBW[a,b] | 6.4 (5.4–7.1) | 6.2 (5.4–7.0) | 6.9 (5.1–7.6) | 0.39 |
| Minute ventilation, L/mn[a,b] | 10.1 (8–12.7) | 10.6 (9.3–13) | 9.0 (7.4–10.5) | 0.11 |
| Plateau pressure, $cmH_2O$[a,b] | 30 (26–32) | 30 (28–33) | 29 (22–32) | 0.27 |
| PaCO2, mmHg[a,b] | 49 (42–57) | 55 (44–58) | 46 (41–55) | 0.33 |
| pH[a,b] | 7.32 (7.22–7.38) | 7.31 (7.22–7.36) | 7.33 (7.26–7.40) | 0.33 |
| $PaO_2/FiO_2$[a,b] | 95 (72–134) | 94 (58–126) | 119 (76–180) | 0.18 |
| Lung injury score[a,b] | 3.0 (2.50–3.25) | 3.25 (2.75–3.25) | 2.65 (2.1–3.1) | 0.049 |
| Compliance, mL/cmH2O[a,b] | 19.6 (15.0–26.6) | 20.0 (15.0–26.0) | 19.0 (15.0–34.0) | 0.90 |
| PEEP, $cmH_2O$[a,b] | 9 (7–12) | 10 (8–12) | 8 (5–9) | 0.045 |
| At least one adjunctive therapy, *n* (%)[b] | 11 (34.4 %) | 8 (42.1 %) | 3 (23.1 %) | 0.45 |
| Death on day 60, *n* (%) | 24 (75) | 16 (84.2) | 8 (61.5) | 0.14 |
| Ventilator-free days at day 60 | 0 (0–5) | 0 (0–0) | 0 (0–35) | 0.029 |

*SOFA* Sequential organ failure assessment [34], *SAPS II* simplified acute physiologic score [35], *ARDS* acute respiratory distress syndrome, *PBW* predicted body weight, *PEEP* positive end-expiratory pressure, *adjunctive therapy* inhaled nitric oxide, almitrine bismesilate, prone positioning, extra-corporeal membrane oxygenation, high-frequency oscillatory ventilation

[a] Median (IQR)

[b] The day of open-lung biopsy

characteristics of these patients are presented in Table 1. The OLB was generally done during the second week of ARDS. Twenty-nine of the patients had an open-lung biopsy during the course of their illness, while a post-mortem lung biopsy was performed in the remaining three patients. One patient presented a moderate air leaks following the OLB. Nineteen patients (59.4 %) presented with a histopathological diagnosis of ARDS-associated lung fibroproliferation. There was a complete agreement between the two pathologists in 29 (90.6 %) of the OLB. Partial agreement was observed in 3 cases (9.4 %) regarding the severity of lung fibroproliferation (mild or moderate). After collegial analysis, the agreement between pathologists was obtained for these 3 cases. The first case was classified as mild fibroproliferation and the 2 others were classified as moderate fibroproliferation.

### *Accuracy of the N-terminal peptide for type III procollagen in diagnosing lung fibroproliferation*

Serum and alveolar NT-PCP-III samples were measured a median of 3 days (1–4 days) prior to lung biopsy. In two patients, BAL was performed with two aliquots of 50 ml of saline (total saline volume administered, 100 ml). Serum and alveolar NT-PCP-III were higher in patients who presented with lung fibroproliferation as compared with patients not presenting with post-aggressive lung fibroproliferation (Fig. 1). Figure 2 shows that the area under the ROC curve for alveolar NT-PCP-III was slightly but not significantly larger as compared to the area for serum NT-PCP-III ($p = 0.12$). The best threshold for alveolar NT-PCP-III was 9 µg/L (Table 2). The best threshold for serum NT-PCP-III was 16 µg/L. The highest diagnostic accuracy for assessing lung fibroproliferation was observed for the alveolar NT-PCP-III (Table 2). Alveolar NT-PCP-III measured by BAL prior to lung biopsy highly correlated with the severity of lung fibroproliferation (Fig. 3) ($p < 0.01$, Rho = 0.635).

## Validation cohort

### *Patients*

Eighty-one consecutive ARDS patients were screened in the three intensive care units (35 beds). Eleven patients

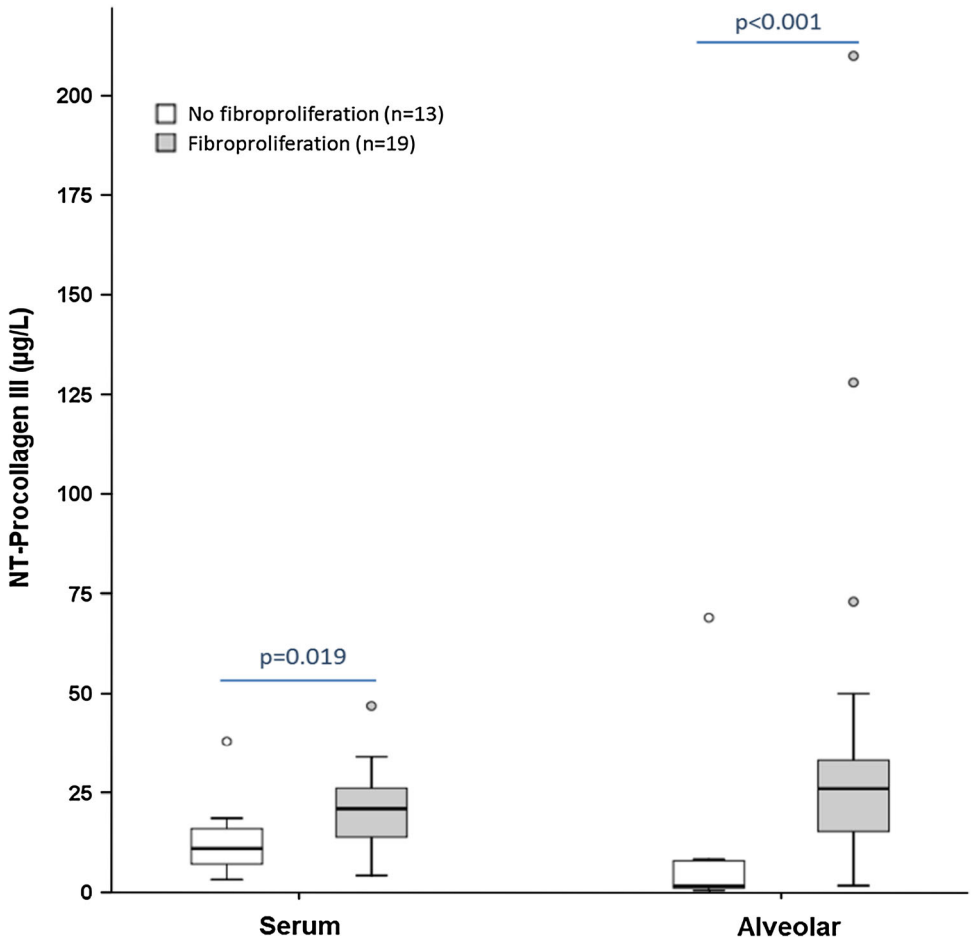


**Fig. 1** Comparisons of serum and alveolar N-terminal peptide for type III procollagen levels according to the presence or not of lung fibroproliferation. *Box plots* represent median (*black bar inside box*), interquartile range (*box*), 10th–90th percentiles (*whiskers*) and outliers plotted separately

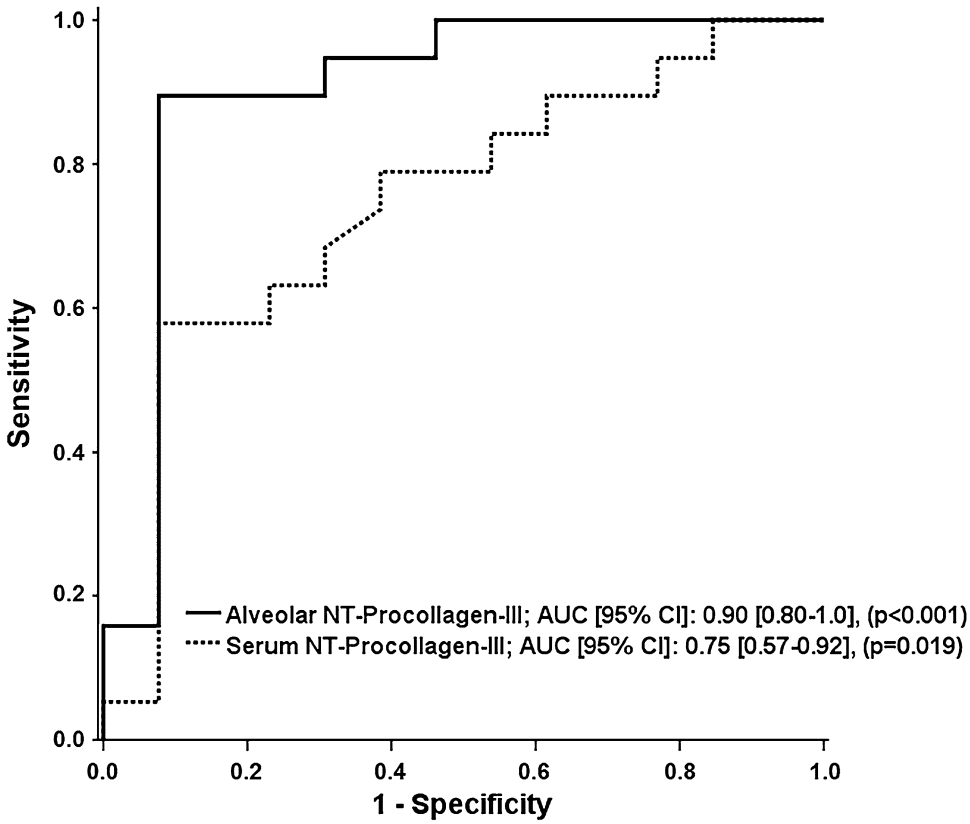


**Fig. 2** ROC curves of serum and alveolar N-terminal peptide for type III procollagen during the week preceding lung biopsy AUC (95 % IC): area under the curve (95 % confidence interval)

**Table 2** Diagnostic performance of serum and alveolar N-terminal peptide for type III procollagen

| | Sensitivity (%) | Specificity (%) | Positive predictive value (%) | Negative predictive value (%) | Diagnostic accuracy (%) | Likelihood ratio positive | Likelihood ratio negative |
|---|---|---|---|---|---|---|---|
| Serum NT-PCP-III >16 μg/L | 63.2 (41.0–80.9) | 76.9 (49.7–91.8) | 80.0 (54.8–92.9) | 58.8 (36.0–78.4) | 68.8 (51.4–82.1) | 2.7 (1.3–5.8) | 0.5 (0.3–0.7) |
| Alveolar NT-PCP-III >9 μg/L | 89.5 (68.6–97.1) | 92.3 (66.7–98.6) | 94.4 (74.2–99.0) | 85.7 (63.1–95.9) | 90.6 (75.8–96.8) | 11.6 (1.6–83.7) | 0.1 (0.04–0.31) |

Value with (95 % CI); NT-PCP-III, N-terminal peptide for type III procollagen

were not included because they presented with exclusion criteria: two refused to participate, two were receiving immunosuppressive therapy, two had liver cirrhosis, two did not meet the safety criteria for BAL ($PaO_2/FIO_2$ <70 mmHg), two presented an advanced directive to withhold life-sustaining treatment, and one was not enrolled due to organizational failure. Of the remaining 70 patients, 54 were still alive and mechanically ventilated on day 7 (16 patients were therefore not included, 8 patients died before day 7 and 8 were weaned from mechanical ventilation before day 7). However, BAL sampling was not done at day 7 for organizational reasons in three patients. Finally, 51 patients were analyzed. In three patients, BAL was performed with two aliquots of 50 ml of saline (total saline volume administered, 100 ml). Their characteristics are shown in Table 3. Direct lung injury was the main mechanism of ARDS (86 % of patients). An open-lung biopsy was performed in 10 of these 51 patients. These 10 patients were also included in the determination part of the study.

### *NT-procollagen III peptide levels in ARDS and the outcome*

As shown in Table 3 and Fig. 4, the mortality rate at day 60 was markedly higher in patients presenting a BAL NT-PCP-III level higher than 9 μg/L as compared with the patients in whom BAL NT-PCP-III was under this threshold (69 vs. 17 % respectively, $p < 0.001$). Figure 5

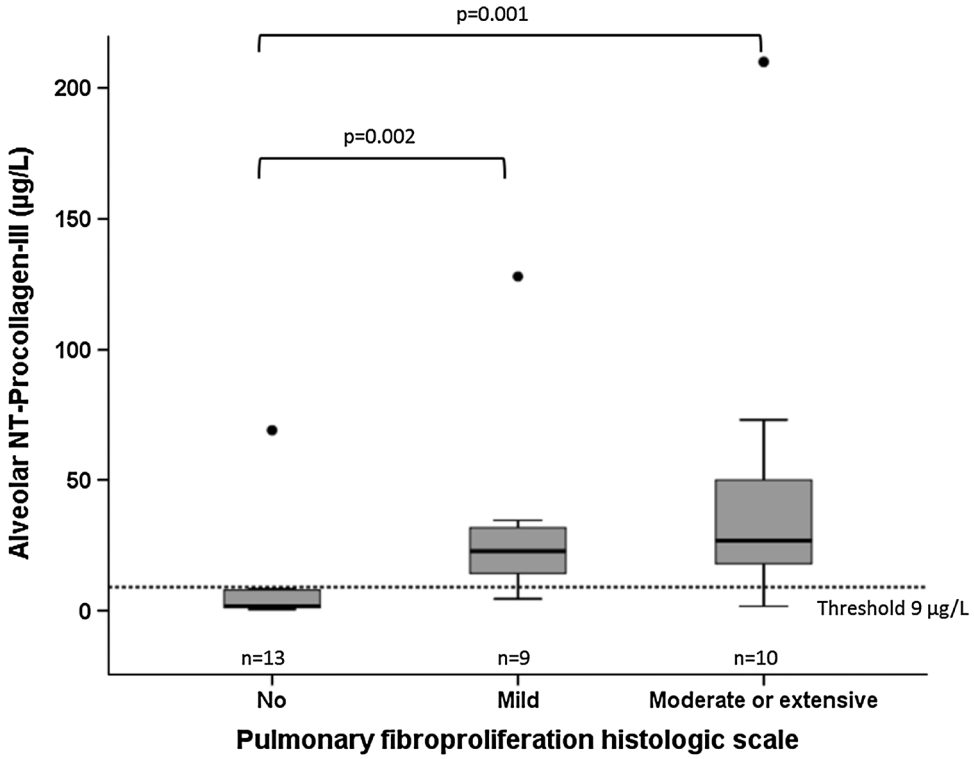


**Fig. 3** Relationship between the alveolar level of N-terminal peptide for type III procollagen and the severity of pulmonary fibroproliferation. Lung fibroproliferation histologic scale: *No* normal lung structure; *Mild* mild interstitial fibroproliferation; *Moderate* moderate interstitial fibroproliferation; and *Extensive* extensive fibroproliferation with lung architecture distortion and alveolar obliteration by dense fibrous material. *Box plots* represent median (*black bar inside box*), interquartile range (*box*), 10th–90th percentiles (*whiskers*) and outliers plotted separately

**Table 3** Validation cohort study: characteristics of the 51 patients

| | All ARDS ($n = 51$) | BAL NT-PCP-III >9 µg/L ($n = 16$) | BAL NT-PCP-III ≤9 µg/L ($n = 35$) | *p* value |
|---|---|---|---|---|
| Age, years | 60 ± 13 | 64 ± 12 | 58 ± 14 | 0.11 |
| Men, *n* (%) | 40 (78) | 15 (94) | 25 (71) | 0.14 |
| SAPS II score on admission | 50 ± 16 | 52 ± 17 | 49 ± 16 | 0.52 |
| SOFA score[a] | 7 ± 5 | 9 ± 4 | 6 ± 5 | 0.12 |
| Cause of ARDS, *n* (%) | | | | 0.11 |
| Pneumonia | 32 (63) | 13 (81) | 19 (54) | |
| Aspiration | 10 (20) | 1 (6) | 9 (26) | |
| Extra-pulmonary infection | 4 (8) | 1 (6) | 3 (9) | |
| Pancreatitis | 1 (2) | 1 (6) | 0 (0) | |
| Miscellaneous | 4 (8) | 0 (0) | 4 (11) | |
| Direct lung injury, *n* (%) | 44 (86) | 14 (88) | 30 (86) | 1.0 |
| $PaO_2/FIO_2$, mmHg[a] | 180 ± 82 | 155 ± 87 | 190 ± 79 | 0.20 |
| Tidal volume, mL/kg PBW[a] | 7.1 ± 1.7 | 6.4 ± 1.4 | 7.4 ± 1.8 | 0.09 |
| Plateau pressure, $cmH_2O$[a] | 25 ± 7 | 26 ± 9 | 24 ± 6 | 0.46 |
| Total PEEP, $cmH_2O$[a] | 11 ± 4 | 13 ± 4 | 10 ± 3 | 0.02 |
| LIS score[a] | 2.2 ± 1.0 | 2.8 ± 1.0 | 1.9 ± 0.9 | 0.01 |
| NT-PCP-III on BAL, µg/L[a] | 17.8 ± 41.5 | 52.3 ± 62.2 | 2.0 ± 2.0 | 0.006 |
| Serum NT-PCP-III, µg/L[a] | 14.5 ± 8.7 | 21.9 ± 9.9 | 11.1 ± 5.5 | 0.001 |
| Death on day 60, *n* (%) | 17 (33) | 11 (69) | 6 (17) | 0.001 |
| Ventilator-free days at day 60 | 27 (0–39) | 0 (0–0) | 36 (0–43) | 0.001 |

Values are expressed as means ± SD or number of cases (%) except for VFD [median (IQR)]

*BAL* broncho-alveolar lavage, *NT-PCP-III* N-terminal peptide for type III procollagen, *ARDS* acute respiratory distress syndrome, *SAPS II* simplified acute physiology score [35], *SOFA* sepsis-related organ failure assessment score [34], $PaO_2/FIO_2$ partial pressure of arterial oxygen/fraction of inspired oxygen ratio, *PBW* predicted body weight; plateau pressure measured during a 1-s end-inspiratory pause, *Total PEEP* total positive end-expiratory pressure measured during a 5-s end-expiratory pause, *LIS* lung injury severity score [36]

[a] On inclusion (day 7)

shows BAL levels of NT-PCP-III according to day 60 mortality. The mean level of NT-PCP-III in BAL on day 7 was 8.1-fold higher in nonsurvivors as compared with survivors ($p = 0.03$). The number of ventilator-free days and alive at day 60 was considerably reduced in the group of patients presenting a BAL NT-PCP-III level higher

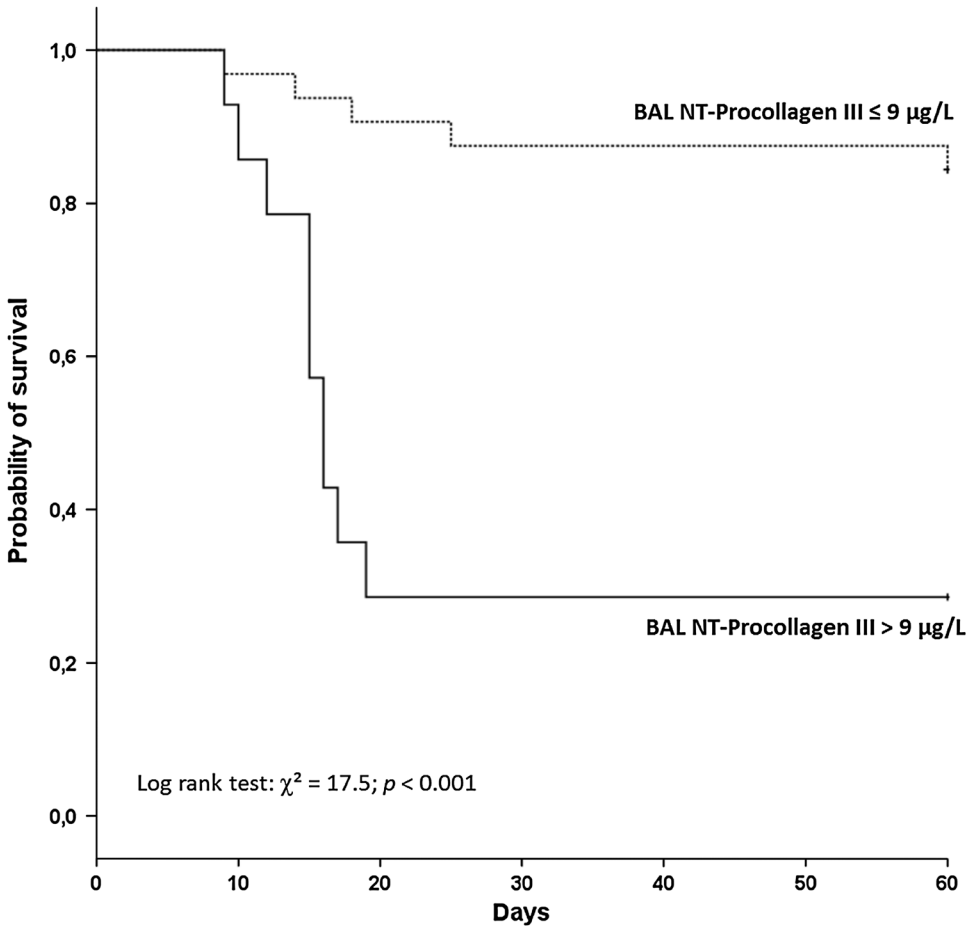


**Fig. 4** Kaplan–Meier curves showing the probability of survival in ARDS patients with high and low bronchoalveolar lavage fluid levels of N-terminal peptide for type III procollagen at day 7

than 9 µg/L (Table 3). As shown in Table 3, the LIS was significantly increased in patients with a BAL NT-PCP-III level higher than 9 µg/L ($2.8 \pm 1.0$ vs. $1.9 \pm 0.9$, $p < 0.01$). The logistic regression analysis including LIS showed that only a NT-PCP-III alveolar level higher than 9 µg/L was independently associated with ICU death [HR (IC95 %) = 5.02 (2.06–12.25), $p < 0.0001$]. Only 3 patients from both groups received corticosteroids for ARDS after day 7.

## Discussion

Measuring alveolar NT-PCP-III during nonresolving ARDS is able to identify patients who have developed lung fibroproliferation.

Type III procollagen has been proposed as a biomarker of lung fibroproliferation in experimental bleomycin models [24, 25]. It has also been validated in patients with idiopathic lung fibroproliferation using histology as the reference method [26, 27]. In contrast, NT-PCP-III has not been validated in ARDS-associated lung fibroproliferation using histology as the gold standard. Histology was only used in a small series of eight patients presenting with pneumonia [16]. In contrast, it has been established that an elevated NT-PCP-III level is associated with a poor outcome [14, 15, 28]. More recently, it has been shown that there is an inverse relationship between the level of NT-PCP-III and the compliance of the respiratory system, suggesting that this biomarker could be a good indicator of lung fibroproliferation in ARDS patients [29].

The present study confirmed the prognostic value of pulmonary levels of NT-PCP-III [14, 15, 28]. We showed that BAL NT-PCP-III levels on day 7 from the onset of ARDS were increased in patients who ultimately died. Chesnutt et al. [15] and Marshall et al. [28] have shown that the alveolar level of NT-PCP-III measured on day 1 from the onset of ARDS was significantly increased in nonsurvivors compared to survivors in 33 and 44 ARDS patients, respectively. Clark et al. [14] analyzed 83 patients on days 3, 7 and 14 from the onset of ARDS and reported higher BAL levels of NT-PCP-III on days 3 and 7 in patients who died compared with survivors.

The use of a sensitive and specific biomarker, such as NT-PCP-III, could be helpful in identifying patients to

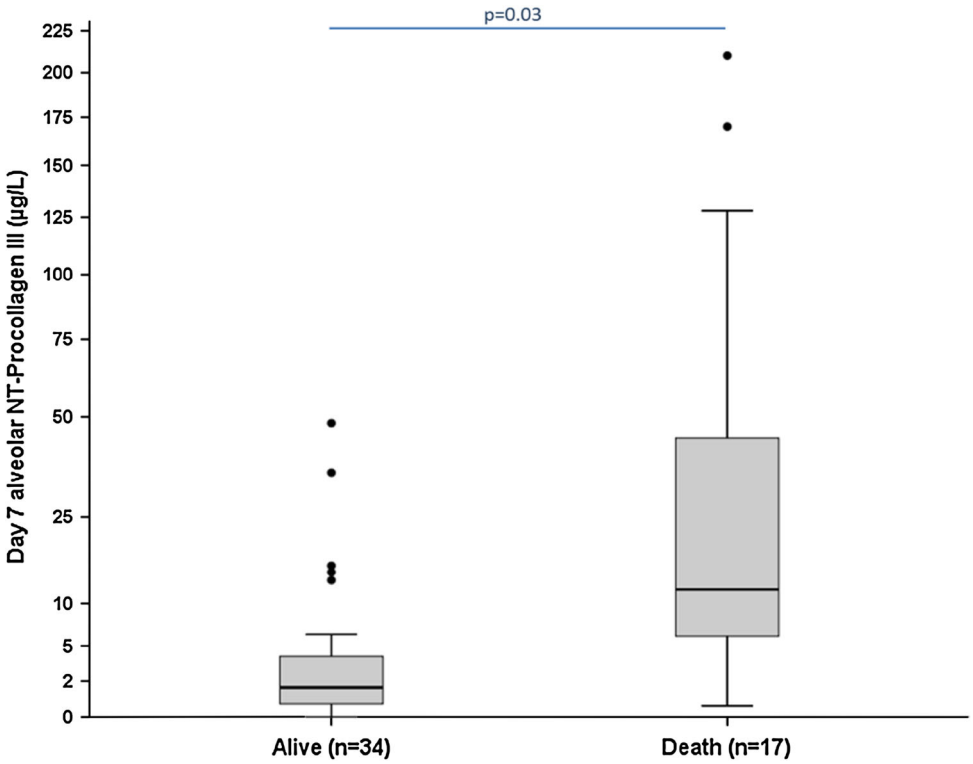


**Fig. 5** Bronchoalveolar lavage fluid levels of N-terminal peptide for type III procollagen at day 7 according to day 60 mortality. *Box plots* represent median (*black bar inside box*), interquartile range (*box*), 10th–90th percentiles (*whiskers*) and outliers plotted separately

include in a trial of corticosteroids or any other treatment that might help resolve lung fibroproliferation. It has been shown that NT-PCP-III decreases in ARDS patients receiving corticosteroids [30]. The randomized-controlled trials on the use of corticosteroids in ARDS have given discordant results [8–10, 31]. In the study from the ARDS network [10], there was no beneficial effect regarding day 60 mortality from using corticosteroids for persistent ARDS. Moreover, there was a suggested detrimental effect with an increased mortality when corticosteroids were given more than 14 days following the onset of ARDS. However, the authors reported a significantly decreased mortality rate among patients with an alveolar NT-PCP-III level greater than the median at baseline [10]. This information strongly suggests that the indication of corticosteroids in persistent ARDS could be guided by NT-PCP-III. It has also been shown that nearly 50 % of open-lung biopsies performed for persistent ARDS did not show any histological sign of fibroproliferation [11]. In this latter study, there were in contrast many infectious diseases not diagnosed by bacterial cultures performed using the BAL. We can speculate that the use of new microbiological tools, such as RT-PCR, in addition to measuring NT-PCP-III in BAL samples, can provide enough information to help clinicians decide whether to give therapies modulating fibroproliferation and avoid the need for an open-lung biopsy [32, 33].

## Study limitations

Lung biopsy specimens and serum/BAL measurements were not performed at the same time. This delay is justified by our usual strategy which is to perform blood and BAL analyses prior OLB and to wait for their results prior to performing OLB. From 2 to 4 days are usually necessary to obtain all microbiological results. Finally, BAL (and blood samples) was done during the first 3 days prior to OLB in 50 % of the cases, and during the first 4 days in 75 % of the cases.

BAL dilution was not assessed and could have modified the present results. Another limitation is that OLB (which is done in a small territory) is not representative of the fibroproliferation process of the entire two lungs. However, one can argue that when fibroproliferation is not seen in a lung territory probably means that the severity of fibroproliferation does not require any additional specific treatment.

Even if this study showed that BAL NT-PCP-III has a high sensitivity and specificity for predicting lung fibroproliferation in non-resolving ARDS patients, further studies will have to be performed to assess whether the subset of patients with an elevated level of NT-PCP-III will benefit from the administration of corticosteroids or any other therapy aiming to modulate fibroproliferation.

## Conclusions

Type III procollagen measure in BAL fluid had a good diagnostic accuracy compared to a histological assessment of fibroproliferation and may be used in future trials of corticosteroids or any other treatment that might help resolve lung fibroproliferation.

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