

# Fibrocytes in Bronchoalveolar Lavage Fluid are associated with Outcome in patients with Acute Lung Injury

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# Background

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Fibrocytes are mesenchymal progenitors from hematopoietic origin involved in normal and pathologic organ repair [1,2]. During acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), an ineffective repair is associated with over-mortality [3]. We investigated whether fibrocyte detection in bronchoalveolar lavage fluid (BALF) could be a predictor of outcome during acute lung injury.

# Methods (1/2)

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- We prospectively collected BAL from 122 ventilated patients (62-ARDS, 30-ALI, 30-ventilated patients w/o ALI/ARDS) as previously described [4]. (Fig. 1)
- Ventilated patients were followed up for 28 days after BAL procedure and clinical data recorded. (Table 1)

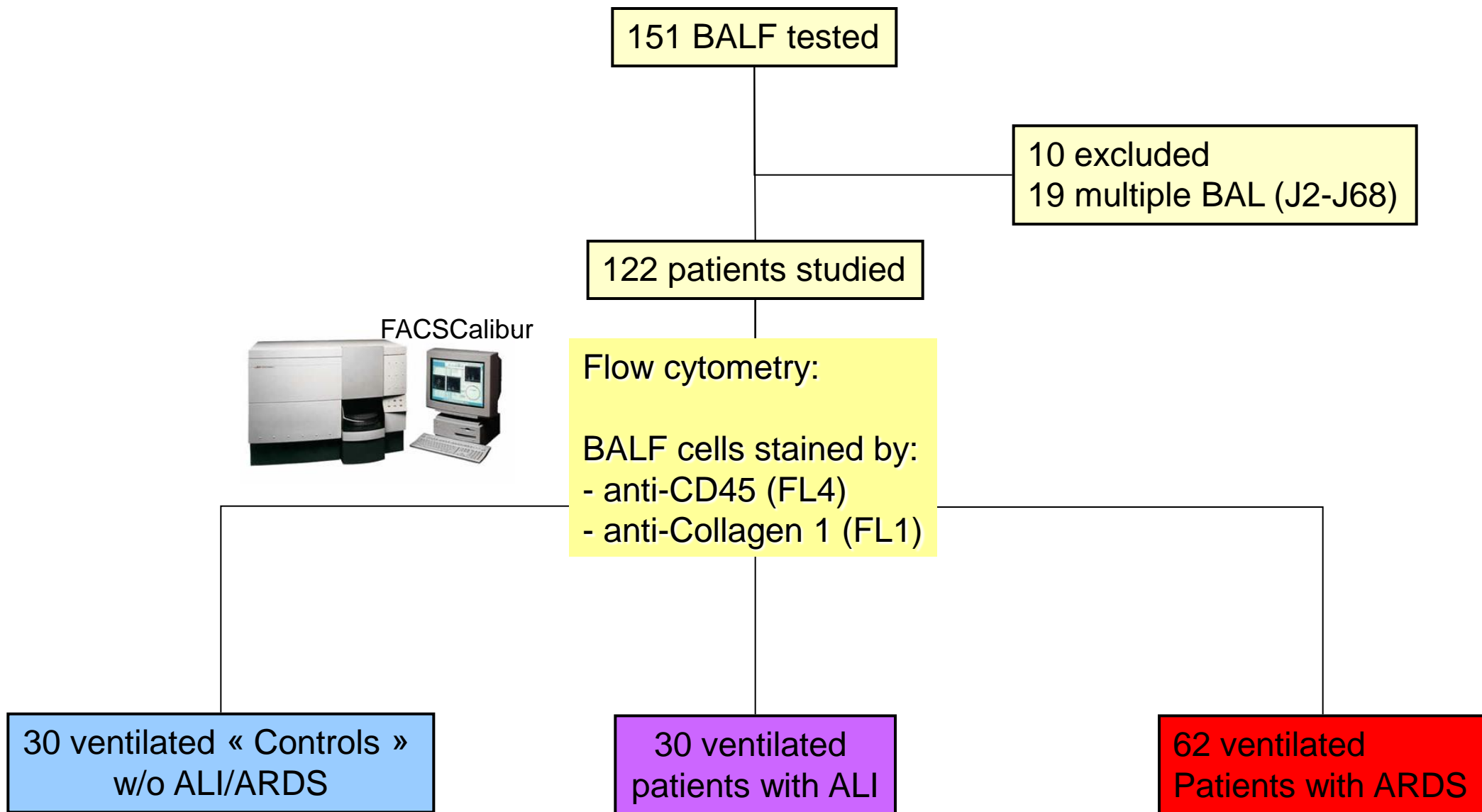


Figure 1- Flowchart

# Table 1- Characteristics of the patients

	Overall (n=122)	w/o ALI/ARDS (n=30)	ALI (n=30)	ARDS (n=62)	p**
<b>At admission:*</b>					
Age, years	67 [54-74]	64 [58-70]	70 [54-77]	67 [49-74]	0.69
Male sex	79 (65)	18 (60)	18 (60)	43 (69)	0.66
SAPS II score †	47 [36-62]	41 [23-60]	50 [38-69]	48 [39-62]	0.03
<b>Comorbidity(ies)</b>					
smoking	48 (39)	14 (47)	7 (23)	27 (44)	0.39
obstructive airway diseases	22 (18)	6 (20)	5 (17)	11 (18)	0.95
cardiovascular disease	20 (16)	11 (37)	4 (13)	5 (8)	0.001
diabetes	32 (26)	9 (30)	9 (30)	14 (23)	0.64
malignancy	25 (20)	5 (17)	8 (27)	12 (19)	0.61
<b>Reason for ICU admission</b>					
sepsis	31 (25)	4 (13)	10 (33)	17 (27)	0.18
pneumonia	26 (21)	1 (3)	4 (12)	21 (34)	0.002
postoperative	28 (23)	12 (40)	4 (12)	12 (19)	0.03
coma	10 (8)	0 (0)	6 (20)	4 (6)	0.01
congestive heart failure	10 (8)	10 (33)	0 (0)	0 (0)	<0.001
hemorrhagic shock	8 (7)	1 (3)	3 (10)	4 (6)	0.58
other	9 (7)	2 (6)	3 (10)	4 (6)	0.82
<b>The day of inclusion:*</b>					
SOFA score ‡	7 [5-9]	7 [5-8]	6 [5-9]	8 [5-10]	0.43
<b>Treatment(s)</b>					
vasopressor	61 (50)	15 (50)	12 (40)	34 (55)	1.00
antibiotics	74 (61)	18 (60)	17 (57)	39 (63)	1.00
sedation	116 (95)	29 (97)	27 (90)	60 (97)	1.00
transfusion	22 (18)	5 (17)	4 (13)	13 (21)	1.00
insulin	119 (98)	29 (97)	29 (97)	61 (98)	1.00
heparin	111 (91)	29 (97)	27 (90)	55 (89)	0.29
hemofiltration	17 (14)	3 (10)	4 (13)	10 (16)	0.56
Surgical patient	62 (51)	18 (60)	17 (57)	27 (44)	0.25
Pulmonary infection	56 (46)	10 (33)	15 (50)	31 (50)	0.14
Duration of mechanical ventilation before inclusion, days	6 [2-12]	4 [1-12]	7 [4-12]	7 [2-12]	0.10
<b>Cause of lung injury in ALI/ARDS (n=92)</b>					
pneumonia	51 (55)	–	15 (50)	36 (58)	0.38
aspiration	10 (11)	–	3 (10)	7 (11)	1.00
sepsis	26 (28)	–	11 (40)	15 (24)	0.23
other	5 (6)	–	1 (0)	4 (7)	1.00
LIS score §	1.7 [1.3-2]	1 [0.7-1.7]	1.3 [1-1.7]	2 [2-2.7]	<0.001
<b>Respiratory variables</b>					
minute ventilation, L/min	9.6 [8.1-11.2]	9.2 [8.1-10.7]	9.5 [7.7-11.2]	9.6 [8.4-11.2]	0.51
respiratory rate, cycles/min	20 [18-22]	19 [17-20]	20 [18-20]	20 [18-24]	0.29
FiO2	0.5 [0.4-0.6]	0.4 [0.4-0.5]	0.4 [0.4-0.5]	0.6 [0.5-0.8]	0.02
PEEP, cmH2O	5 [5-7]	5 [5-5]	5 [5-6]	6 [5-8]	0.004
PaO2/FiO2	186 [62-500]	247 [190-306]	239 [210-282]	140 [107-167]	<0.001
PaO2, mmHg	86 [75-113]	99 [82-149]	102 [86-119]	78 [66-91]	0.01
PaCO2, mmHg	41 [34-48]	38 [32-42]	41 [34-46]	42 [35-53]	0.02
arterial pH	7.4 [7.33-7.44]	7.4 [7.34-7.44]	7.4 [7.36-7.42]	7.4 [7.3-7.44]	0.68
<b>Outcome parameters:*</b>					
Mortality 28 days after inclusion	41 (34)	4 (13)	11 (37)	26 (42)	0.02
Total duration of mechanical ventilation among survivors, days	16 [8-30]	14 [7-22]	15 [11-28]	22 [9-40]	0.18
Length of stay in ICU, days	19 [10-35]	17 [7-42]	25 [14-31]	20 [9-35]	0.45

\* values are medians and [IQR] or number and (%). Because of rounding, percentages may not total 100. ALI denotes Acute Lung Injury, ARDS Acute Respiratory distress syndrome, ICU Intensive Care Unit, FiO2 fraction of inspired oxygen, PaO2 partial pressure of arterial oxygen, PaCO2 partial pressure of arterial carbon dioxide, and PEEP positive end-expiratory pressure.

† SAPS II denotes the Simplified Acute Physiology Score; scores can range from 0 to 163, with higher scores indicating more severe illness.

‡ SOFA denotes the Sequential Organ Failure Assessment; scores can range from 0 to 24, with higher scores indicating more organ failures.

§ LIS denotes the Lung Injury Scores can range from 0 to 4, with higher scores indicating more severe injury.

\*\* Comparison between patients w/o ALI/ARDS (n=30) and ALI/ARDS (n=92)

# Methods (2/2)

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- Fibrocytes, defined as cells expressing CD45 and collagen 1, were quantified by flow cytometry. (Fig. 2)
- Chemokines, growth factors and collagen 1 concentrations were measured in BAL supernatants by ELISA method. (Table 2)

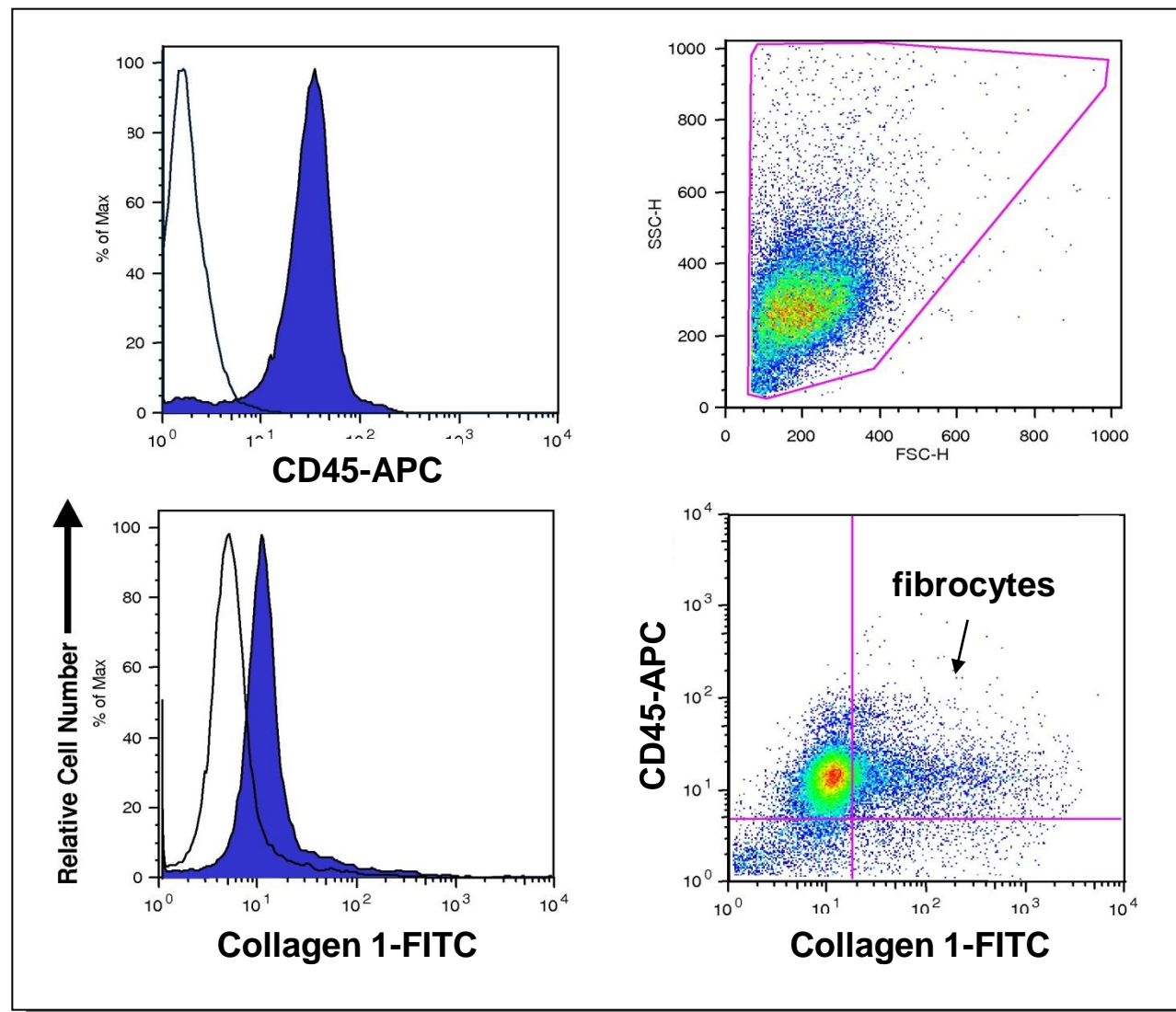


Figure 2- Identification of a double stained cell population (CD45+/collagen1+) in BALF

# Table 2- Characteristics of BALF from patients

	Patients Mechanically Ventilated			p**
	w/o ALI/ARDS (n=30)	ALI (n=30)	ARDS (n=62)	
BALF protein, g/L	0.14 [0.11-0.45]	0.30 [0.20-0.57]	0.36 [0.18-0.49]	0.02
BALF/serum protein ratio	0.002 [0.002-0.009]	0.006 [0.003-0.011]	0.006 [0.003-0.009]	0.05
Total cell count, cells/mm <sup>3</sup>	172 [50-676]	461 [89-2652]	327 [100-1471]	0.03
Neutrophil, %	53 [13-90]	79 [63-90]	87 [70-92]	0.004
Mononuclear cells, %	47 [10-87]	21 [10-37]	13 [8-30]	0.004
Fibrocytes, %	0.9 [0-3.4]	5.0 [2.9-9.1]	5.0 [3.2-8.8]	<0.001
CXCL8 (pg/ml)	814 [469-3357]	2433 [1337-5854]	2460 [716-7442]	0.03
CXCL12 (pg/ml)	<5 [<5-31]	17 [<5-39]	<5 [<5-27]	0.55
CCL2 (pg/ml)	61 [31-152]	266 [70-475]	147 [40-390]	0.03
CCL19 (pg/ml)	<5 [<5-29]	12 [<5-58]	6 [<5-37]	0.12
CCL21 (pg/ml)	<10 [<10-136]	<10 [<10-275]	<10 [<10-278]	0.41
HGF (pg/ml)	150 [<40-406]	685 [76-1392]	396 [190-1030]	0.001
TGF $\beta$ -1 (pg/ml)	10 [<5-34]	21 [8-45]	15 [<5-44]	0.08
proCollagen 1 (pg/ml)	<200 [<200-525]	<200 [<200-2825]	<200 [<200-6013]	0.34

\* values are medians and [IQR]. ALI denotes Acute Lung Injury, ARDS Acute Respiratory distress syndrome, BALF Bronchoalveolar lavage fluid Intensive Care Unit, FiO<sub>2</sub> fraction of inspired oxygen, CXCL8 chemokine (C-X-C motif) ligand 8, CXCL12 chemokine (C-X-C motif) ligand 12, CCL2 Chemokine (C-C motif) ligand 2, CCL19 Chemokine (C-C motif) ligand 19, CCL21 Chemokine (C-C motif) ligand 21, HGF Hepatocyte Growth Factor, TGF $\beta$ -1 Transforming Growth Factor beta-1. \*\* Comparison between patients w/o ALI/ARDS (n=30) and ALI/ARDS (n=92)



# Results (1/2)

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- Fibrocytes were detected in 89% of BAL from ventilated patients. (Fig. 3)
- The median percentage of BAL fibrocytes was significantly increased in patients with ALI and ARDS (5.0 %) in comparison with ventilated controls (0.9%,  $p < 0.001$ ). (Fig. 3)
- Fibrocyte percentage correlated with percentage of monocytes/macrophages in BAL ( $r = 0.35$ ,  $p = 0.007$ ). None other biological factor was significantly associated with the percentage of fibrocytes in BAL from ALI/ARDS patients.

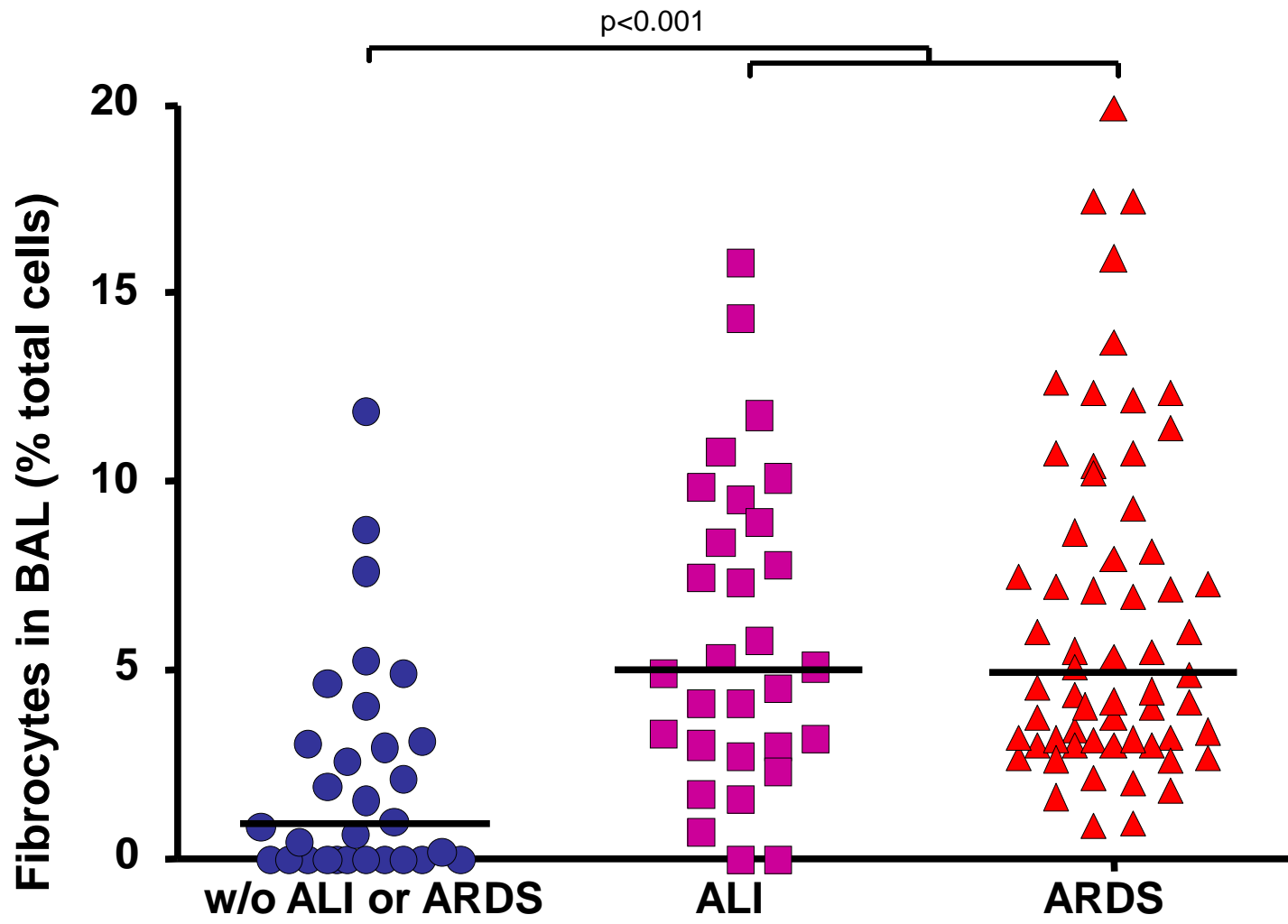
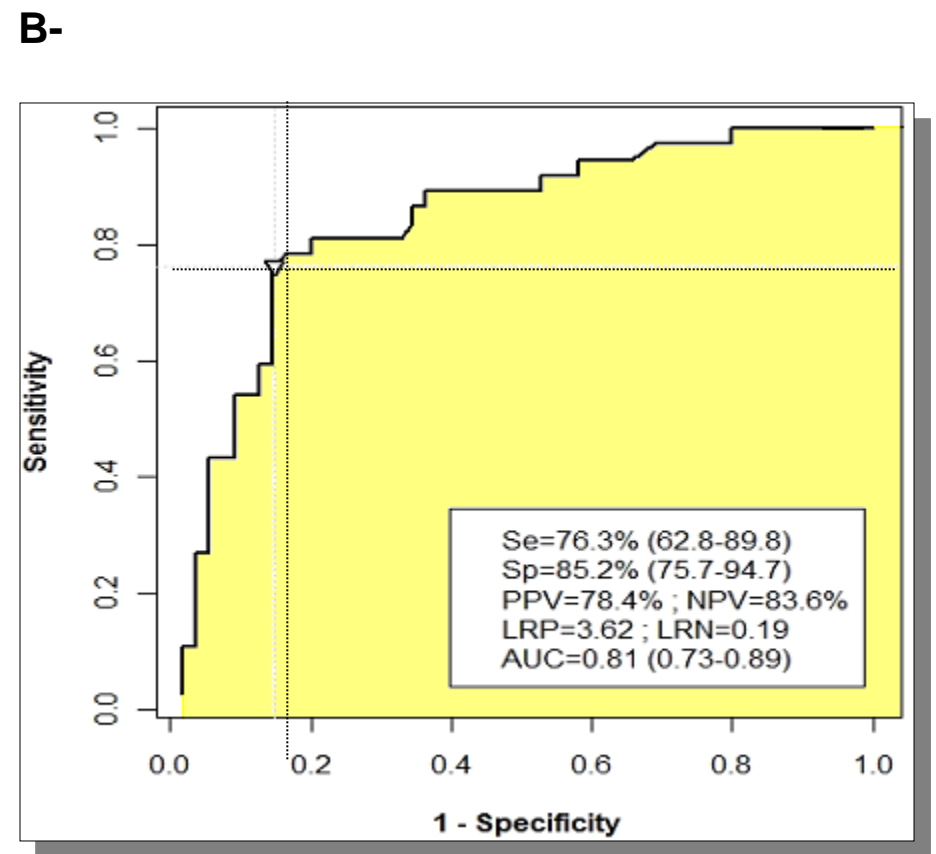
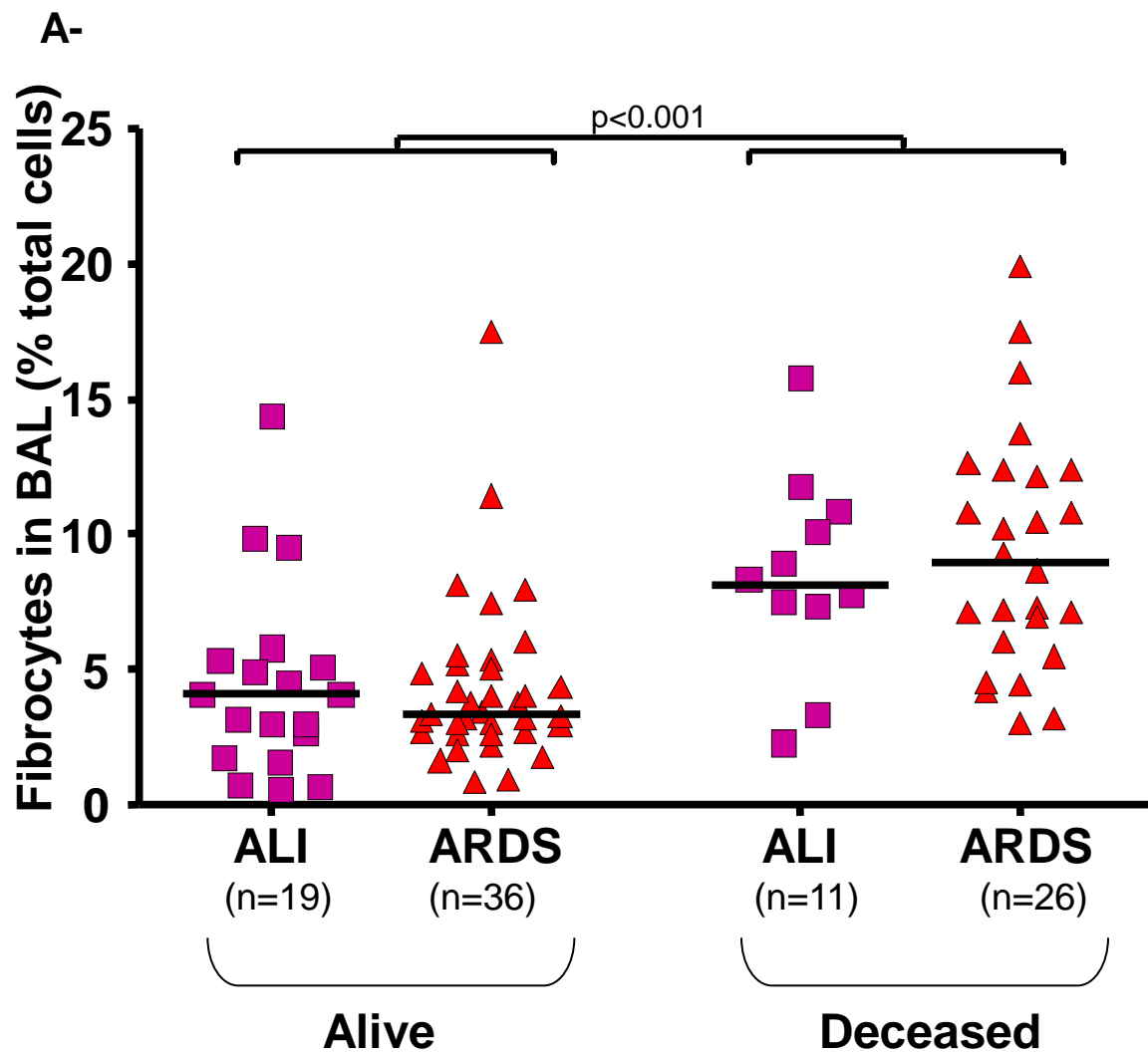


Figure 3- Percentage of fibrocytes in BALF

# Results (2/2)

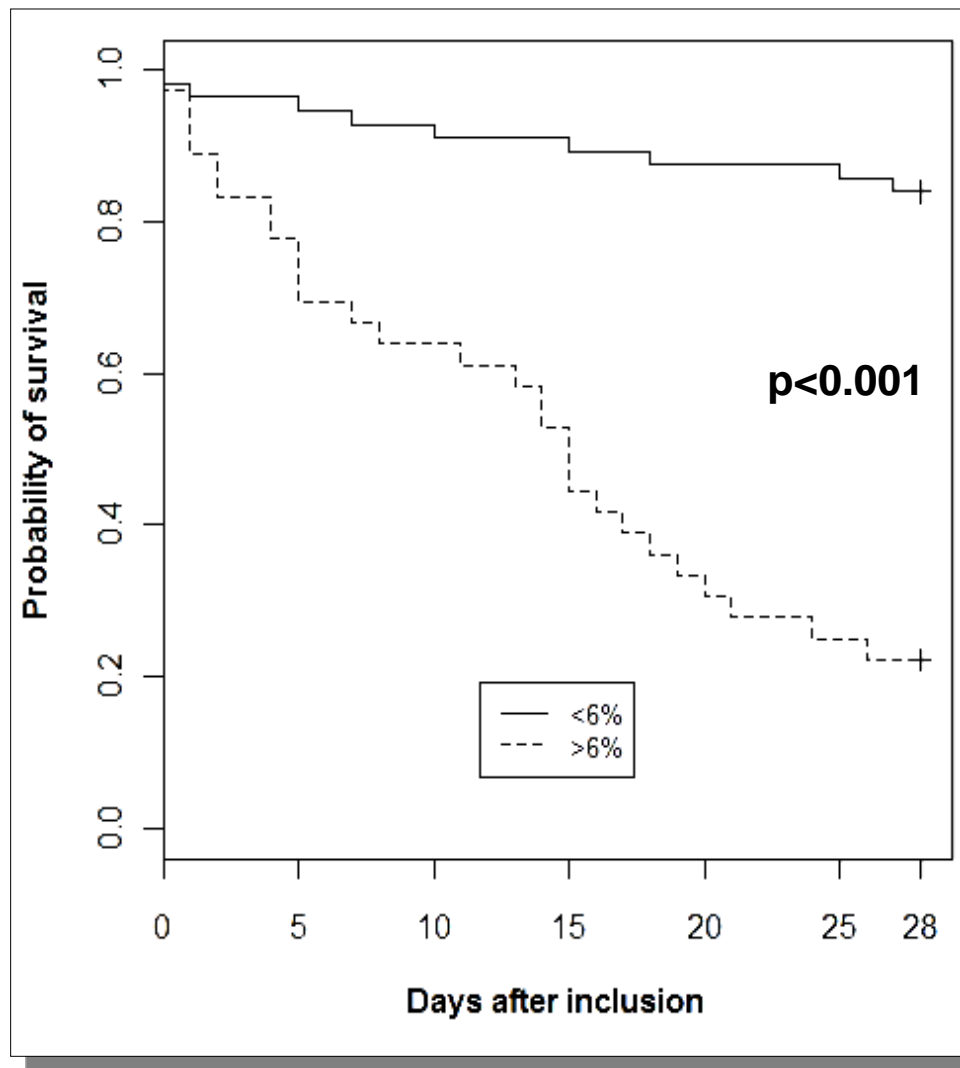
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- In ALI/ARDS patients (n=92), the median BAL fibrocyte percentage from non-survivors was 2.5 fold higher than in those from survivors (8.65 vs. 3.41 %, respectively ;  $p < 0.001$ ). (Fig. 4A)
- After adjustment for age, comorbidities, administered treatments and severity of illness in a multivariable COX proportional-hazard model, a percentage of BAL fibrocytes over 6% (Fig. 4B) was independently associated with a lower proportion of patients weaned from mechanical ventilation at 28 days (HR [95% CI]= 0,34 [0,15-0,78],  $p = 0,01$ ) and a higher 28-day mortality in ALI/ARDS patients (HR [95% CI]= 6.15 [2.78-13.64],  $p \leq 0.0001$ ). (Fig 5., Table 3)



ROC curve analysis:  
 → Optimal cut-off value to discriminate  
 Alive and deceased patients is equal to 6%

Figure 4- Percentage of fibrocytes in BALF is associated with mortality during ALI/ARDS



Patients with ALI/ARDS were separated in a group of patients with more than 6% fibrocytes (dotted line, n = 36) and a group with less than 6% fibrocytes (solid line, n = 56).

Figure 5- Survival curves of patients with ALI/ARDS (n=92).

# Table 3: Cox Proportional Hazard Analysis of Predictors of Mortality in Patients with ALI/ARDS

Variable	Univariate Cox Model		Multivariate Cox Model	
	HR (95% CI)	p	HR (95% CI)	p
<b>At admission:</b>				
Age, /block of 10 years	1.04 [1.11-1.77]	0.005	1.37 [1.08-1.75]	0.01
SAPS II score	1.02 [1.00-1.04]	0.01	NA	–
Comorbidity of malignancy	2.63 [1.33-5.18]	0.005	4.14 [1.81-9.47]	<0.001
<b>The day of inclusion:</b>				
SOFA score, /point	1.23 [1.12-1.35]	<0.001	1.27 [1.13-1.44]	<0.001
Vasopressor infusion	3.23 [1.59-6.54]	0.001	NA	–
Transfusion	2.66 [1.33-5.32]	0.006	NA	–
Hemofiltration	2.86 [1.38-5.93]	0.005	NA	–
Pulmonary infection	0.49 [0.25-0.96]	0.04	NA	–
FiO2	1.02 [1.00-1.03]	0.01	NA	–
PaO2, mmHg	1.01 [1.00-1.02]	0.01	NA	–
<b>BAL characteristics:</b>				
Neutrophils, % in BAL	0.98 [0.97-0.99]	0.003	NA	–
Percentage of fibrocytes in BAL, > 6%	8.20 [3.83-17.6]	<0.001	6.15 [2.78-13.64]	<0.001
TGF $\beta$ -1, pg/ml BAL fluid	0.99 [0.98-1.00]	0.02	NA	–

NA: not appropriate; ALI denotes Acute Lung Injury, ARDS Acute Respiratory distress syndrome, FiO2 fraction of inspired oxygen, PaO2 partial pressure of arterial oxygen, TGF $\beta$ -1 Transforming Growth Factor beta-1, SAPS II denotes the Simplified Acute Physiology Score, SOFA denotes the Sequential Organ Failure Assessment, BAL Bronchoalveolar lavage

# Conclusion

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- Fibrocytes can be detected in 89% of BAL from ventilated patients.
- The BAL fibrocyte percentage is significantly increased in ventilated patients with ALI/ARDS as compared to ventilated controls
- BAL Fibrocytes percentage  $>6\%$  is an independent marker associated with poor survival and requirement for prolonged mechanical ventilation in patients with ALI/ARDS.
- After validation on a second cohort of patients, this biological marker could be useful in clinical strategy.

# References

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