Lung Biopsy in Acute Respiratory Distress Syndrome

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Introduction

The etiological diagnosis of pulmonary infiltrations on chest radiography in intensive care unit (ICU) patients remains an everyday problem, especially in cases of acute respiratory distress syndrome (ARDS) where numerous factors can come into play. In terms of survival, Meduri et al. demonstrated the potential benefits of anti-inflammatory treatment instituted in the late stages of ARDS during the fibroproliferative phase [1]. However, the benefits of this approach require differentiation of an infection and the beginning of fibrosis. Current microbiological examinations (tracheal aspiration, protected specimen brush [PSB], bronchoalveolar lavage [BAL]) lack sensitivity and/or specificity for the diagnosis of infection. The unsuitable character of these examinations for the diagnosis of fibrosis, despite the possibility of dosing serum and alveolar biological markers of fibrosis, such as procollagen III, justify performing histologic and microbiologic analysis of lung parenchyma samples obtained by biopsy.

After a discussion of the pathologic lesions found in ARDS, we will attempt to define the indications for lung biopsy in patients with ARDS. We will describe the different sampling techniques used, as well as the treatment and analysis of samples. We will conclude with the clinical consequences of such research and propose an attitude for ICU practice.

ARDS: Histological Aspects

The mechanisms of ARDS development are only partially known despite the fact that they have been studied for over 20 years. These mechanisms differ according to whether the ARDS is primary (direct aggression of the alveoli) or secondary (indirect systemic aggression). Despite these physiopathologic differences, the anatomopathologic abnormalities encountered are quite similar. In fact, during ARDS, there is a succession, or even coexistence, of three phases that can be distinguished histologically.

Early Phase

In the early phase (or exsudative phase), an intraalveolar and interstitial exudate made up of plasmatic proteins, fibrin, and inflammation cells essentially with polynuclear neutrophils and eosinophils, will appear in the first hours and persist for at least one week [2]. During this phase, the alveolar surfactant is altered by a modifi-

cation in its composition and its functional capacities (alteration of its tensioactive properties). At this stage, there can already be associated morphological lesions of the alveolocapillary barrier with stripping of the endothelial and epithelial basal membrane. Cellular destruction therefore involves the type I epithelial cells (cellular lysis and/or apoptosis) rather than the endothelial cells. The intensity of the epithelial lesions is recognized as an important prognostic factor in ARDS [3]. Macroscopically, the lung has a 'heavy' appearance, edematous or even hemorrhagic, with the section showing a consolidated parenchyma.

Fibro-proliferative Phase

This interstitial and alveolar exudate is then infiltrated by macrophages and fibroblasts. Various activated proteases (elastase, neutrophils, gelatinases) will damage the basal membrane and the extracellular matrix. This so-called organization (or proliferation) phase is characterized by the proliferation of type II pneumocytes, endothelial cells and fibroblasts. The latter have the morphological characteristics of myofibroblasts. Fibroblast proliferation is mediated by signaling molecules (fibronectin, collagen fragments, fibrin, elastin), growth factors and tumor necrosis factor (TNF)-α. Alveolar collapse occurs in the most severely damaged regions with the occasional appearance of real endoalveolar buds. From a macroscopic point of view, the lung has a pale gray color and a smooth surface.

Vascular Lesions. During these first two phases there is increased pulmonary capillary permeability (in part responsible for the exudate) as well as a reduction in the number and the volume of these capillaries [4]. Microthrombi are found with the presence of fibrinoplatelet clusters in the arterioles and capillaries associated with fibrin clots in the pre-acinary and acinary arterioles. At the level of the pulmonary artery, the presence of thrombi appears to be the rule as seen in an autopsy study where this type of lesion was found in 95% of the cases [5]. The vascular structural modification, which can subsequently occur (microcirculatory fibrous occlusion, smooth muscle hypertrophy), thereby contributes to pulmonary arterial hypertension, which is frequently described in ARDS and is associated with poor outcome.

Fibrosis Phase

The proliferation phase can evolve toward fibrosis, a factor of poor outcome [6]. Such fibrosis was found in 40% of the cases in a series of open-lung biopsies performed in 36 patients with ARDS [7]. Fibrosis is formed by an anarchic endoalveolar and septal deposit of type III collagen, then type I, secreted by the fibroblasts. Alveolar and capillary spaces are therefore obstructed, considerably disturbing pulmonary architecture and function. At this stage, the edema has usually disappeared. In addition, there is intraalveolar angiogenesis in response to excessive growth factor production. Macroscopically, the lung has a rough appearance, with a pale and spongiform appearance in sections. Numerous cystic areas and fibrous scars are often observed. Fibrosis can be detected beginning on the fifth day of ARDS. The process most certainly begins earlier given the presence of procollagen III (precursor of collagen synthesis) at elevated levels in the alveolar liquid after 24 hours of evolution [8, 9]. Elevated plasma and alveolar procollagen III levels appear to be predictive markers for ARDS mortality [10, 11]. However, to our knowledge,

no study has correlated procollagen levels with the presence of fibrosis confirmed by lung biopsy.

These three phases are, in reality, superimposed in space and time, thereby creating a great heterogeneity of pulmonary lesions in the same patient.

Lung Biopsy Indications in ARDS

There are two essential reasons that move a clinician to perform or prescribe lung parenchyma sampling within the framework of ARDS:

- Define an etiology that is potentially curable when less invasive examinations such as BAL have not been conclusive,
- Reveal the signs of fibrosis in order to prescribe a possible anti-inflammatory corticoid treatment that could contribute to the chances for survival [1].

Such potentially immunosuppressive corticotherapy is not without risk. In pulmonary infection, microbiologic sampling techniques can lack sensitivity or specificity [12–15], particularly for virus, to contraindicate or delay corticotherapy after beginning an adapted anti-infectious treatment. A series of 36 patients with ARDS who had undergone surgical pulmonary biopsy had cytomegalovirus (CMV) in 50% of the cases [7]. Moreover, biologic analysis of alveolar liquid by dosage of certain markers such as cytokines, alveolar proteins, procollagen III for diagnosis of fibrosis is not yet in current practice.

Lung biopsy could, therefore, be indicated when initial examinations are not conclusive, when ARDS is not evolving favorably after five days (no reduction in Lung Injury Score [LIS] [16]) or earlier if a neoplasic etiology is suspected. The absolute or relative contraindications, hemorrhagic, hemodynamic, or respiratory risks must be taken into account according to the technique under consideration. The risk of transporting a patient to the operating room if the procedure is not possible at the bedside in the ICU must also be evaluated.

Lung Biopsy Techniques

Transparietal Biopsies

Transparietal (percutaneous) biopsy can be performed under fluoroscopy, tomodensitometry, or ultrasound with a specific needle. The major risk of pneumothorax in a ventilated patient makes this technique inadvisable, particularly in ARDS patients. In addition, the yield of this technique in the framework of a diffuse process is very debatable given the small sample size. However, this type of sampling has a place in peripheral radiological opacity exploration, especially in thoracic oncology.

Transbronchial Biopsies

Transbronchial biopsy is performed under bronchial endoscopy (flexible fiberscope or rigid endoscope) directed or not by fluoroscopy. Four to seven biopsies are performed in the same territory to provide for both microbiologic and histologic analyses. This is an attractive technique for pneumologists because it is usually practiced with a flexible fiberscope in awake patients under local anesthesia. A few

studies have demonstrated the feasibility and safety of this technique in patients under mechanical ventilation [6, 17, 18]. Simple measures must therefore be taken during this procedure: increase of FiO_2 to 1, discontinuation of positive end-expiratory pressure (PEEP) and rise of maximum pressure alarms. In a recent study of 38 mechanically ventilated patients [19], all with unexplained pulmonary infiltration, BAL and transbronchial biopsy were successively performed. They provided a diagnosis in 74% of the subjects and led to a modification in treatment in 63% of the cases. Transbronchial biopsy alone ensured a diagnosis in 63% of the cases whereas BAL alone provided a diagnosis in 29%. In the subgroup of late-phase ARDS patients (n = 11), the diagnosis was established in 64% of the cases by associating both diagnostic methods: fibroproliferation was found in all of the cases, leading to prescription of corticoids. An examination of the various studies on the subject reveals that transbronchial biopsy provides a diagnosis in an average of 40% of the cases. Nevertheless, the yield of this technique is controversial given the small size of the samples or their low numbers, which sometimes make a quality histological examination difficult [7]. In a study of 116 transbronchial biopsies, Fraire et al. reported a correlation between the number of fragments obtained, the number of alveoli per fragment (more than 20) and the specificity of the histologic results in the diagnosis of infectious pneumonia [20]. A study comparing transbronchial biopsy with open-lung biopsy in 20 patients with chronic pulmonary infiltration demonstrated the superiority of the surgical technique with a diagnosis in 94% of the cases for surgical biopsy and 59% with transbronchial biopsy [21]. However, there are still some arguments in favor of the latter, such as the simplicity of its performance and the possibility to repeat the examination more easily than in the case of surgical biopsy. The risks of this technique in addition to those inherent to bronchial endoscopy are essentially pneumothorax, pneumomediastinum, and hemoptysis. The lateral bronchi of the basal pyramid appear to offer less risk in terms of pneumothorax [22]. In cases of diffuse pulmonary pathology, the American Thoracic Society does not recommend systematic use of fluoroscopy since it did not appear to modify yield or the risks of this procedure in a non-randomized prospective study [23]. The risk of hemoptysis is reduced by ensuring that the coagulation results and platelet count are compatible with the procedure. An old prospective series of 5450 transbronchial biopsies performed in non-ventilated patients reported a mortality rate of 0.25% (13 deaths, including 9 attributable to hemorrhagic complications, the majority with hemostasis problems). This same study showed the need for pneumothorax drainage in 5.5% of the cases [24]. Studies performed in ventilated patients have reported a mean incidence of 12% for pneumothorax (requiring drainage) without persistent bronchopleural fistula [6, 17, 18, 25, 26] and of 11% for moderate bleeding.

Surgical Biopsies

Surgical lung biopsies are performed by video-assisted thoracoscopy or by thoracotomy. With ARDS, only biopsy by thoracotomy (open-lung biopsy) can be performed since it is impossible to do the selective intubation required for surgical thoracoscopy. These open-lung biopsies can be performed at the bedside in the ICU. This technique is of great interest for patients who can only be transported to the operating room with great difficulty given their frequent hemodynamic and respiratory instability.

Technique. Anticoagulants must be stopped, when possible, at least six hours before the biopsy. The surgeon and the intensivist together must choose the side for sampling. The choice is preferably the most pathologic side unless pleural symphysis is suspected or in case of a history of lung resection on the same side. These elements are evaluated from the patient's history, chest x-ray and, if necessary, thoracic CT scan. During the biopsy, FiO_2 is set at 1. The patient is positioned in the supine position. A pad is positioned along the scapula homolateral to the biopsy in order to tilt the patient approximately 15°. The homolateral upper limb is held in abduction with the forearm folded up over the head. A portable monopolar electrocoagulator is essential to complete hemostasis. An anterolateral thoracotomy of approximately 10 cm is performed in the fifth intercostal space. Pleural liquid (serofibrinous effusion is present in two-thirds of the patients) is sampled for cytologic and microbiologic analysis. Extreme care must be taken in handling the pulmonary tissue in order to avoid alveolar rupture. Particular attention is given to hemostasis. A single but broad biopsy is performed in an area that appears to be macroscopically pathologic. It is most often performed at the level of the lingula, from the middle or the lower lobes. The biopsy is performed with mechanical forceps with linear stapling. The ventilator must be temporarily disconnected in order to limit tissue thickness to ensure better aerostasis. The parenchymatous sample is then cut into four parts and each fragment is packaged in a specific manner for histologic, bacteriologic, virologic, and parasito-mycologic analyses. Two drains – an anterior and a posterior – are inserted. The mean duration of the procedure is 30 minutes. Verification by chest X-ray is performed at the end of the procedure.

Monitoring. Monitoring is essentially clinical and radiological. Air leaks are evaluated, blood loss is evaluated daily and the surgical wound is regularly examined for any local complications. Chest X-ray is performed daily until the drains are taken out which is usually done after weaning from the ventilator.

Complications. It appears that no cases of peroperative death directly attributable to the biopsy have been reported in the literature [7, 27–30]. The postoperative complications generally reported are prolonged air leak (15 to 20% of cases) [7, 28, 30]. Evolution, most often spontaneously favorable, can require mobilization of the thoracic drains or placement of a new pleural drain. Other complications such as parenchymatous or pleural infections or hemorrhages are rarely observed [7, 30]. Table 1 summarizes the results concerning the ventilatory conditions and complications encountered in series of surgical pulmonary biopsies [7, 27, 28, 31–34].

Packaging, Preparation and Analysis of the Samples

Packaging of the samples. The transbronchial biopsies must be immediately fixed in formalin 10% or in Bouin liquid. It is unadvisable to fragment this type of biopsy owing to the risk of damaging it and making morphologic analysis impossible. The minimum duration of fixing in formalin is usually short for small samples for which the time of sampling must be noted. Once fixed, the sample is handled conventionally by an anatomopathologic laboratory: dehydration, inclusion in paraffin, and preparation of several histologic sections stained with hematoxylin-eosin. Depending on the conventional morphologic aspect and the clinical context, the analysis can be completed by immunohistochemical study of specific antibodies. On average, the histologic results of a biopsy are available 24 hours after the sampling.

Table 1. Comparison of seven series of surgical biopsies

	n	Place	PEEP	PaO_2/FiO_2	Complications
Hill [31]	42	ICU	6.5 (0–15)	84 (30–350)	1 bubbling 1 hemothorax 1 infection
Ashbaugh [32]	10	?	10–20	42–74	0
Warner [33]	20	OR	?	?	?
Meduri [27]	7	OR	?	?	?
Canver [28]	27	OR	9 ± 0.8	?	6 bubbling 2 pneumothorax
Meduri [34]	12	OR	?	?	1 bubbling
Papazian [7]	37	ICU (25) OR (12)	5–16	79–304	1 pneumothorax 1 hemothorax

OR: operating room

Table 2. Principal staining used for biopsies

Staining	Principal infectious agents
Gram/Giemsa	Bacteria
Gomori-Grocott	Fungal agents (pneumocystis, aspergillus, histoplasma…)
PAS (Periodic Acid Schiff)	Fungal agents
Ziehl	Mycobacteria

For morphologic analysis of transbronchial biopsy, three whole biopsy fragments are sent directly to the different laboratories (bacteriology, virology and parasitomycology) in dry sterile jars for staining according to the clinical orientation (Table 2), direct immunofluorescence for legionella and culture on bacteriologic, virologic and mycologic media.

Surgical lung biopsies performed by thoracotomy require, for optimal results, good coordination of the intensivist, the surgeon and the pathologist. In order to avoid contamination, samples for infectious purposes are ideally sent directly from the operating room or the ICU to the microbiology laboratory in dry sterile jars where they are processed like the transbronchial biopsy. Samples for histologic purposes are sent to the anatomopathologic laboratory at a cool temperature in a dry flask with a screwed top and without the addition of a liquid. Samples must therefore be delivered as soon as possible – within a maximum of one hour following the sampling. On reception and according to the clinical context communicated by the intensivist, specific analyses for microbiologic purposes can then be performed on the surgical specimen. Fragments can be frozen in nitrogen for molecular biology study or immunofluorescence or be placed in glutaraldehyde for study by electronic microscopy. The rest of the surgical specimen is fixed in formalin 10%. If the biopsy is small, it is always better to favor conventional morphology and fix the entire sample in formalin. The fixed fragment is processed in a conventional manner in an anatomopathologic laboratory like the transbronchial biopsy.

Clinical Consequences

The clinical consequences of pulmonary biopsy performed within the framework of ARDS can be major. Therapeutic modifications can be prescribed according to the anatomopathologic and/or microbiologic results: corticotherapy in case of fibroproliferation or fibrosis; prescription or modification of anti-infectious treatment, particularly in the framework of a cytomegalovirus or herpes infection [7]. In the same manner, a prognosis can be totally changed by these results: discovery of a potentially curable etiology, diagnosis of primary or secondary lung cancer (carcinomatous lymphangitis essentially in the framework of ARDS), fibrosis, which could benefit from anti-inflammatory treatment, worsening the vital prognosis. The time period before a specific treatment is administered (particularly corticotherapy in case of fibrosis) can be shortened by the carrying out of a rapid analysis before fixing and a more detailed analysis of the principal specimen, with a small fragment immediately taken to an anatomopathologic laboratory. In the same manner, immediate analysis makes it possible to avoid prescription of potentially deleterious corticotherapy if signs of infection, particularly viral, are detected. In a series of 36 patients [7] where prescription of corticotherapy was considered before surgical biopsy, only 40% (15 patients) really had an indication for anti-inflammatory treatment on receipt of the anatomopathologic results owing to the presence of fibroproliferation or fibrosis. Only six patients benefited from such treatment straightaway – the nine others had CMV on biopsy.

Conclusion

We propose a practical attitude to the diagnosis and specific treatment of ARDS, based on lung biopsy. BAL is used to look for etiology and anti-infectious treatment is prescribed if pulmonary infection is suspected. If ARDS persists after 7–10 days of evolution despite an adapted treatment and if the BAL is negative, surgical biopsy is undertaken and followed by specific treatment once the results are known.

References

1. Meduri GU, Headley AS, Golden E, et al (1998) Effect of prolonged methylprednisolone therapy in unresolving acute respiratory distress syndrome: a randomized controlled trial. JAMA 280:159–165
2. Ware LB, Matthay MA (2000) The acute respiratory distress syndrome. N Engl J Med 342:1334–1349
3. Ware LB, Matthay MA (2001) Alveolar fluid clearance is impaired in the majority of patients with acute lung injury and the acute respiratory distress syndrome. Am J Respir Crit Care Med 163:1376–1383
4. Tomashefski J (2000) Pulmonary pathology of acute respiratory distress syndrome. Clin Chest Med 21:435–466
5. Tomashefski J, Davies P, Boggis C, Zapol W, Reid L (1983) The pulmonary vascular lesions of the adult respiratory distress syndrome. Am J Pathol 112:112–126
6. Martin C, Papazian L, Payan MJ, Saux P, Gouin F (1995) Pulmonary fibrosis correlates with outcome in adult respiratory distress syndrome: a study in mechanically ventilated patients. Chest 107:196–200
7. Papazian L, Thomas P, Bregeon F, et al (1998) Open-lung biopsy in patients with acute respiratory distress syndrome. Anesthesiology 88:935–944

8. Marshall RP, Bellingan G, Webb S, et al (2000) Fibroproliferation occurs early in the acute respiratory distress syndrome and impacts on outcome. Am J Respir Crit Care Med 162:1783–1788
9. Chestnutt AN, Matthay MA, Tibayan FA, Clark JG (1997) Early detection of type III procollagen peptide in acute lung injury. Pathogenetic and prognostic significance. Am J Respir Crit Care Med 156:840–845
10. Clark JG, Milberg JA, Steinberg KP, Hudson LD (1995) Type III procollagen peptide in the adult respiratory syndrome. Ann Intern Med 122:17–23
11. Meduri GU, Tolley EA, Chinn A, Stentz F, Postlehwaite A (1998) Procollagen types I and III aminoterminal propeptide levels during acute respiratory distress syndrome and in response to methylprednisolone treatment. Am J Respir Crit Care Med 158:1432–1441
12. Torres A, El-Ebiary M, Padro L, et al (1994) Validation of different techniques for the diagnosis of ventilator-associated pneumonia. Comparison with immediate postmortem pulmonary biopsy. Am J Respir Crit Care Med 149:324–331
13. Marquette CH, Georges H, Wallet F, et al (1993) Diagnostic efficiency of endotracheal aspirates with quantitative bacterial cultures in intubated patients with suspected pneumonia. Am Rev Respir Dis 148:138–144
14. Chastre J, Fagon JY, Bornet-Lesco M (1995) Evaluation of bronchoscopic techniques for the diagnosis of nosocomial pneumonia. Am J Respir Crit Care Med 152:231–240
15. Papazian L, Thomas P, Garbe L, et al (1995) Bronchoscopic or blind bronchial sampling techniques for the diagnosis of ventilator-associated pneumonia. Am J Respir Crit Care Med 152:1982–1991
16. Murray JF, Matthay MA, Luce JM, Flick MR (1988) An expanded definition of the adult respiratory distress syndrome. Am Rev Respir Dis 138:720–723
17. Papin TA, Grum CM, Weg JG (1986) Transbronchial biopsy during mechanical ventilation. Chest 89:168–170
18. Pincus PS, Kallenbach JM, Hurwitz MD, et al (1987) Transbronchial biopsy during mechanical ventilation. Crit Care Med 15:1136–1139
19. Bulpa PA, Dive AM, Mertens L, et al (2003) Combined bronchoalveolar lavage and transbronchial lung biopsy: safety and yield in ventilated patients. Eur Respir J 21:489–494
20. Fraire AE, Cooper SP, Greenberg SD, Rowland LP, Langston C (1992) Transbronchial lung biopsy. Histopathologic and morphometric assessment of diagnostic utility. Chest 102:748–752
21. Burt ME, Flye MW, Webber BL, Path FF, Wesley RA (1981) Prospective evaluation of aspiration needle, cutting needle, transbronchial and open biopsies in chronic infiltrate lung diseases. Ann Thorac Surg 32:146–151
22. Dierkesmann R, Dobbertin I (1998) Different techniques of bronchoscopy. Pulmonary endoscopy and biopsy techniques. Eur Respir Monogr 9:1–21
23. Anders GT, Johnson JE, Bush BA, Matthews JI (1988) Transbronchial biopsy without fluoroscopy. A seven-year survey. Chest 94:557–560
24. Herf SM, Surrat PM, Arora NS (1977) Deaths and complications associated with transbronchial lung biopsy. Am Rev Respir Dis 115:708–711
25. O'Brien JD, Ettinger NA, Shevlin D, Kollef MH (1997) Safety and yield of transbronchial biopsy in mechanically ventilated patients. Crit Care Med 25:440–446
26. Turner JS, Willcox PA, Hayhurst MD, Potgieter PD (1994) Fiberoptic bronchoscopy in the intensive care unit. A prospective study of 147 procedures in 107 patients. Crit Care Med 22:259–264
27. Meduri GU, Belenchia JM, Estes RJ, Wunderink RG, El Torky M, Leeper Kv Jr (1991) Fibroproliferative phase of ARDS. Clinicals findings and effects of steroids. Chest 100:943–952
28. Canver CC, Mentzer RM Jr (1994) The role of open lung biopsy in early and late survival of ventilator-dependent patients with diffuse lung disease. J Cardiovasc Surg 35:151–155
29. McKenna RJ Jr, Mountain CF, Mc Martney MJ (1984) Open lung biopsy in immunocompromised patients. Chest 86:671–674
30. Flabouris A, Myburgh J (1999) The utility of open lung biopsy in patients requiring mechanical ventilation. Chest 115:811–817
31. Hill JD, Ratliff JL, Parrot JC, et al (1976) Pulmonary pathology in acute respiratory insufficiency: lung biopsy as a diagnostic tool. J Thorac Cardiovasc Surg 71:64–71

32. Ashbaugh DG, Maier RV (1985) Idiopathic pulmonary fibrosis in adult respiratory distress syndrome. Diagnosis and treatment. Arch Surg 120:530–535
33. Warner DO, Warner MA, Divertie MB (1988) Open lung biopsy in patients with diffuse pulmonary infiltrates and acute respiratory failure. Am Rev Respir Dis 137:90–94
34. Meduri GU, Chinn AJ, Leeper KV, et al (1994) Corticosteroid rescue treatment of progressive fibroproliferation in late ARDS. Patterns of response and predictors of outcome. Chest 105:1516–1527

Cardiac Crises