Diagnostic value of BAL fluid cellular profile and enzymes in infectious pulmonary disorders

N.A.M. Cobben*, J.A. Jacobs**, M.P. van Dieijen-Visser***, P.G.H. Mulder†,
E.F.M. Wouters*, M. Drent*

ABSTRACT: Determination of the cellular profile of bronchoalveolar lavage fluid (BALF), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) appeared to be useful in monitoring pulmonary damage. The aim of this study was to investigate whether the cellular profile, LDH, its isoenzyme pattern and/or ALP in BALF are useful in the diagnostic work-up of patients with suspected pneumonia.

The BALF specimens of 80 patients were studied. Group I consisted of patients with a pulmonary infection (n=33) and group II of patients without signs of a pulmonary infection (n=47). Differentiation between these two groups was based upon the results of microscopy and quantitative cultures.

The absolute as well as relative numbers of polymorphonuclear neutrophils (PMNs) was significantly higher in group I compared to group II (p<0.0001). The absolute number of PMNs showed a sensitivity of predicting the correct group of 95.7% and a specificity of 84.8%. The LDH activity in BALF was significantly higher in group I than in group II (p<0.0001). The LDH4/LDH5 ratio in BALF was lower in group I compared to group II (p<0.0001) and appeared to be the best discriminator between the two groups with a sensitivity of 93.6% and a specificity of 93.9%.

In conclusion, the number of polymorphonuclear neutrophils as well as the lactate dehydrogenase activity, particularly its isoenzymes, in bronchoalveolar lavage fluid appeared to be of potential practical value to distinguish between infectious and noninfectious pulmonary disorders.

activity in serum as well as in BALF [10]. Lung parenchymal cells and/or local inflammatory cells, including AMs and PMNs, may be potential sources of LDH in BALF.

It has been previously demonstrated that the LDH isoenzyme pattern differed between BALF samples with mainly PMNs (high LDH, and BALF samples with mainly AMs (high LDH, ) [11]. As a consequence, the LDH/LDH ratio appeared to be significantly lower in BALF samples with predominantly PMNs compared to BALF samples with mainly AMs.

The aim of the present study was to evaluate whether the cellular profile and/or enzyme activity, e.g. ALP, LDH and its isoenzymes in BALF, have additional practical value in the diagnostic work-up of patients with suspected pneumonia to distinguish between samples of an infectious and noninfectious aetiology.

Methods

General experimental design

The study was conducted at the University Hospital Maastricht, the Netherlands, from February 1996 to January 1998. Eighty BALF samples from hospitalized patients were used for this study. The indication for the lavage varied. Mostly, a pulmonary infection or a diffuse interstitial lung disease was suspected. Exclusion criteria were BALF recovery <35 mL and contamination with red blood cells and/or oropharyngeal cells. Additionally, these 80 BALF samples were divided into two groups: group I consisted of BALF samples obtained from patients with a confirmed pulmonary infection (n=33) (based on culture results ≥10³ cfu·mL⁻¹) and group II consisted of BALF samples from patients without signs of pulmonary infection (n=47) (based on negative culture results <10³ cfu·mL⁻¹ and the absence of intracellular bacteria). The positive culture results of the BALF samples obtained from group I were: Haemophilus influenzae (n=7), Staphylococcus aureus (n=6), Pseudomonas aeruginosa (n=6), Escherichia coli (n=2), Proteus mirabilis (n=2), Streptococcus pneumoniae (n=1), Klebsiella pneumoniae (n=1), K. oxytoca (n=1), Citrobacter diversus (n=1), P. aeruginosa and P. mirabilis (n=1), S. aureus and H. influenzae (n=1), S. pneumoniae and Neisseria meningitidis (n=1), E. coli and K. oxytoca (n=1), E. coli and H. influenzae (n=1), P. mirabilis and H. influenzae (n=1). The patients of group II suffered from: drug-induced interstitial lung disease and pulmonary fibrosis (n=16), acute respiratory distress syndrome (ARDS) developed for several reasons (n=4), pulmonary manifestation of malignancy (n=3), sarcoidosis (n=2), cardiac failure and pulmonary oedema (n=2), lung contusion after trauma (n=3), immunocompromised disorders with chest radiograph abnormalities (n=5), chemical pneumonitis after aspiration (n=2) and no diagnosis (n=10). The majority of cases from the infectious group (n=25) were intubated and mechanically ventilated patients from the intensive care unit (ICU). In the group of noninfectious disorders, 26 of the cases were patients from the hospital ward and the other 25 cases were intubated and mechanically ventilated patients from the ICU. A group of eight healthy volunteers without a relevant medical history, was used as a control group.

Bronchoalveolar lavage

BAL was performed as reported previously during fibroptic bronchoscopy [12]. The procedure is briefly described. If the patient was intubated, the bronchoscope was introduced through the tube. Otherwise, BAL was performed by standardized washing of the involved lobe with four aliquots of 50 mL sterile saline (0.9% NaCl) at 37°C after premedication (0.5 mg atropine intramuscularly and occasionally 5–10 mg diazepam orally), and local anaesthesia of the larynx and bronchial tree (lidocaine 0.5%). Upon arrival in the laboratory, the recovered volume of the BALF was recorded. The first fraction (bronchial fraction) was discarded and the remaining fractions were pooled. After mixing, the BALF was split into two portions, portion one was immediately sent to the department of clinical chemistry and portion two was used for cytological and microbiological analysis. The total cell count was performed in a Fuchs-Rosenthal haemocytometer chamber (Optik Labor Emergo, Landmeer, the Netherlands).

Cyto centrifugation was performed with the Shandon Cytospin 3 apparatus (Shandon Scientific Ltd., Astmoor, UK) (31,000 ×g, 10 min, at room temperature low acceleration rate). In order to obtain monolayer preparations, the number of drops per preparation was adjusted according to the total cell count. The preparations were air dried and subsequently stained according to the May-Grünwald Giemsa (MGG) and Gram’s staining methods. The differential cell count of the MGG-stained preparations was performed by one observer counting 500 nucleated cells. The number of cells containing intracellular organisms was expressed as a percentage of all nucleated cells counted [13]. In this study, BALF samples containing excessive amounts of red blood cells, squamous epithelial and/or ciliated cells, background debris or damaged nucleated cells were excluded from analysis. In addition, BALF samples demonstrating P. carinii cysts were excluded. Quantitative bacterial cultures were performed on appropriate media incubated both aerobically and anaerobically. Mycobacterial and fungal cultures were performed on all BALF samples. Cultures for viruses and Legionella spp. were performed, when clinically indicated. For infectious aetiology, BALF samples were defined as those samples with a quantitative culture yielding ≥10⁴ cfu·mL⁻¹. Regarding noninfectious aetiology, BALF samples were categorized if standard bacterial cultures yielded micro-organisms in quantities <10³ cfu·mL⁻¹ and if other cultures failed to reveal any pathogen and the absence of intracellular organisms at microscopic examination.

Laboratory tests

In the second portion of the BALF samples, chemical analyses including LDH, LDH isoenzymes, ALP, total protein and albumin were also performed. The LDH activity was measured at 37°C by an enzymatic rate method, using pyruvate as a substrate. The test was performed on a Beckman Synchron CX-7 analyser (Beckman Instruments Inc, Mijdrecht, the Netherlands) with Beckman reagents (testkit-442660) and was optimized according to the recommendations of the Deutsche Gesellschaft für Klinische Chemie (DGKC-recommendations).
The LDH activity was expressed in micromoles of substrate (p-nitrophenylphosphate) per minute (U), per litre of serum, at 37°C. The measuring range is 10–1,800 U L⁻¹, for concentrations of 1,800–3,800 U L⁻¹ the samples were automatically diluted with saline and reanalysed, while manual dilution was performed for higher concentrations. Total protein and albumin concentrations are summarized in table 3. The per-  

The characteristics and serum laboratory results of the two groups studied are summarized in table 1. The cellular profile, ALP, LDH and LDH isoenzyme activities in BALF samples of infectious and noninfectious aetiology were examined. The cellular characteristics of BALF of the studied groups are given in table 2. The enzyme and protein concentrations are summarized in table 3. The per-

Recovery mL

PMNs %

AMs %

Lymphocytes %

Lymphocytes × 10⁸ cells mL⁻¹

Eosinophils %

Eosinophils × 10⁶ cells mL⁻¹

Mast cells %

Mast cells × 10⁴ cells mL⁻¹

1. Statistical evaluation

Data are expressed as mean±SEM. In order to detect statistically significant differences between the two patient groups, for each explanatory variable separately, the Mann-Whitney U-test was used. Logistic regression was used to test the discriminatory effect of explanatory variables simultaneously. In these analyses, likelihood ratio tests were used; variables with a significance >10% were left out of the logistic regression models. The results are presented by means of receiver operation characterics (ROC) curves [15].

Results

Data are expressed as mean±SEM. AMs: alveolar macrophages; PMNs: polymorphonuclear neutrophils. *: p<0.05, **: p<0.01, ***: p<0.001, all group I versus group II; : p<0.05, : p<0.01, ***: p<0.001 all versus control group.
The percentage of AMs was significantly lower in the infectious group (7.0±1.3%) compared to the noninfectious group (47.4±3.7%; p<0.0001). The absolute number as well as the percentage of PMNs were significantly higher in the infectious group (309.6±81.3 and 90.5±1.5%) compared to the noninfectious group (5.5±1.2 and 21.6±3.4%; p<0.0001 and p<0.0001, respectively).

Between both patient populations with disorders of infectious and noninfectious aetiology, the ALP activity, albumin and total protein revealed no significant differences (table 3). However, compared to the control group, these parameters were significantly higher in both studied groups (table 3). The LDH activity in the BALF of group I (infectious aetiology) was significantly higher (662±125 U.L⁻¹) compared to group II (147±22 U.L⁻¹; p<0.0001). Moreover, the LDH isoenzyme pattern differed between both groups, particularly LDH2 (table 3). The LDH3/LDH5 and the LDH4/LDH5 ratios were significantly lower in the BALF samples of infectious aetiology patients (0.27±0.02 and 0.44±0.02) compared to the noninfectious BALF samples (1.57±0.20 and 1.43±0.12; p<0.0001). In serum, LDH was elevated in the infectious group (727±50 U.L⁻¹) as well as in the noninfectious group (1026±262 U.L⁻¹) compared to the control group (p<0.001), but no significant difference was found between both groups, respectively (table 1).

When using only the absolute number of PMNs per millilitre of fluid, the sensitivity of predicting the correct group was 100 (45/47)=95.7%, with a lower specificity of 100 (28/33)=84.8% (cutoff point 23.7±10⁴ mL⁻¹)(figs.1 and 2). As shown in figure 3, with respect to the enzyme activity, logistic regression analysis revealed the best discrimination between both groups using the LDH4/LDH5 ratio with a sensitivity of 100 (44/47)=93.6% and a specificity of 100 (31/33)=93.9% (cutoff point 0.60). A LDH4/LDH5 ratio <0.50 was indicative for a noninfectious nature of the BALF, at the cost, however, of a lower sensitivity of 100 (33/47)=70.2%. In contrast, a LDH4/LDH5 ratio >0.80 pointed to a noninfectious nature of the BALF with a 100% specificity, at the cost, however, of a lower sensitivity of 100 (33/47)=70.2%.

Table 3. – Lactate dehydrogenase (LDH), percentage of LDH isoenzymes, alkaline phosphatase (ALP), total protein and albumin in bronchoalveolar lavage fluid (BALF) samples of group I (infectious aetiology), group II (noninfectious aetiology) patients and of healthy control subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (n=33)</th>
<th>Group II (n=47)</th>
<th>Control group (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein mg.L⁻¹</td>
<td>614±155</td>
<td>646±163</td>
<td>28±3</td>
</tr>
<tr>
<td>Albumin mg.L⁻¹</td>
<td>297±97</td>
<td>344±96</td>
<td>17±2</td>
</tr>
<tr>
<td>ALP U.L⁻¹</td>
<td>55±10</td>
<td>45±7</td>
<td>7±2</td>
</tr>
<tr>
<td>LDH U.L⁻¹</td>
<td>662±125</td>
<td>147±22</td>
<td>64±4</td>
</tr>
<tr>
<td>LDH₁ %</td>
<td>3.0±0.3</td>
<td>8.6±0.9</td>
<td>14.5±2.1</td>
</tr>
<tr>
<td>LDH₂ %</td>
<td>6.9±0.5</td>
<td>15.6±0.8</td>
<td>23.1±1.5</td>
</tr>
<tr>
<td>LDH₃ %</td>
<td>13.7±0.6</td>
<td>25.2±0.7</td>
<td>29.1±0.8</td>
</tr>
<tr>
<td>LDH₄ %</td>
<td>22.1±0.5</td>
<td>26.4±0.6</td>
<td>22.9±1.7</td>
</tr>
<tr>
<td>LDH₅ %</td>
<td>54.3±1.5</td>
<td>24.0±1.6</td>
<td>10.5±0.9</td>
</tr>
<tr>
<td>LDH₃/LDH₅</td>
<td>0.27±0.02</td>
<td>1.57±0.20</td>
<td>2.86±0.24</td>
</tr>
<tr>
<td>LDH₄/LDH₅</td>
<td>0.44±0.02</td>
<td>1.43±0.12</td>
<td>2.21±0.15</td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM. *: p<0.0001 group I versus group II; #: p<0.05; **: p<0.005 and ###: p<0.0001 all versus control group.
infectious nature of the BALF with a specificity of 100% and a sensitivity of 100 (28/33)=84.8%, respectively. Using the LDH₄/LDH₅ ratio, a sensitivity of 100 (45/47)=95.7% and a specificity of 100 (29/33)=87.9% was found. The smoking history was of no influence on the results.

The majority of cases from group I (n=25, 76%) were intubated and mechanically ventilated patients from the ICU. In group II, 55% (n=26) of the cases were patients from the hospital ward and the other 45% (n=25) were intubated and mechanically ventilated patients from the ICU. Statistical analyses were performed between these subgroups. Although these results (data not shown) demonstrate that there is a difference between intubated and nonintubated cases in both groups, the nonintubated cases of group I still had a significantly higher number of PMNs as well as a lower LDH₄/LDH₅ ratio in BALF compared to the intubated patients from group II.

In the group of noninfectious BALF samples, four patients were diagnosed as having ARDS. In this latter subgroup, the absolute number (5.56±3.66 × 10⁴ cells·mL⁻¹) and relative number of PMNs (30±13%), the LDH (168±54 U·L⁻¹) and LDH₄/LDH₅ ratio (1.15±0.34) in BALF were also significantly different from the BALF samples of the infectious aetiology group.

Discussion

This study demonstrated that the cellular profile of BALF samples of infectious aetiology appeared to be significantly different from samples of noninfectious aetiology. In particular, the absolute and relative number of PMNs were significantly higher in the infectious group. Furthermore, the LDH activity in BALF was higher in the infectious group compared to the noninfectious group. More specifically, the LDH isoenzyme pattern differed between both groups, of which LDH₃ was the most prominent. The best discriminator between both groups appeared to be the LDH₄/LDH₅ ratio. The ALP activity, albumin and total protein concentrations in BALF revealed no significant differences between both groups.

Cellular bronchoalveolar lavage fluid profile

In addition to their defensive role, PMNs and AMs have recently been implicated in injurious processes associated with both acute and chronic pulmonary diseases [16]. In the normal lung, AMs are resident cells, whereas PMNs are usually absent. However, in certain conditions, PMNs can accumulate within the lung structures [1, 16]. Until now, little attention has been paid to the usefulness of this particular cell type in distinguishing BALF samples of infectious aetiology from noninfectious aetiology. Previously, Kirbyland et al. [17] found that <50% PMNs in BALF had a 100% negative predictive value for histoplastic pneumonia. Marquette et al. [18] also found increased PMNs in patients with pneumonia (87±13%) in comparison to patients without pneumonia (49±32%). In agreement with these findings, the present study found a high total cell count as well as increased numbers of PMNs in the BALF samples of the infectious aetiology group compared to the group of noninfectious aetiology. Moreover, analysis of the cellular profile appeared to be useful in identifying other causes of the pulmonary damage such as fibrosis, drug-induced pneumonitis, diffuse alveolar dam-

Lactate dehydrogenase activity in bronchoalveolar lavage fluid

The enzyme activity in BALF may provide a quantitative assessment of cell damage and pulmonary defence mechanisms. As mentioned previously, not only the amount of cells involved in an inflammatory response are of importance, but also the activity reflected by the release of inflammatory mediators or enzymes indicating cell damage or death such as LDH and ALP [23]. Many studies in animals reported the relationship between LDH activity and pulmonary disorders [23, 24]. In humans, high serum LDH activity was found in several pathological pulmonary conditions, such as pulmonary embolism, P. carinii pneumonia, tuberculosis, bacterial pneumonia [25], diffuse interstitial pneumonitis, extrinsic allergic alveolitis [26], drug-induced respiratory distress [20], lipoid pneumonia [19], idopathic pulmonary fibrosis [26, 27] and silicosis [28]. Furthermore, LDH activity in sputum appeared to be useful to differentiate lower respiratory tract infections from other clinical entities [29]. The LDH and ALP activity has been found to be higher in BALF with mainly PMNs compared to BALF with predominantly AMs [11]. This finding was consistent with the higher inflammatory response indicated by the PMNs [30]. In line with this, in the present study, the number of PMNs as well as the LDH activity was higher in the BALF samples of infectious aetiology. Furthermore, in a previous study [11], the LDH isoenzyme pattern in BALF samples with predominantly PMNs differed from BALF samples with mainly AMs, mainly due to a high LDH₅ level, and as a consequence a lower LDH₄/LDH₅ ratio. In the present study, a different LDH isoenzyme pattern in the group of BALF samples of infectious aetiology compared to the samples of noninfectious aetiology was found. Comparable with the higher amount of PMNs in the infectious group, a different LDH isoenzyme pattern was also found, with a lower LDH₄/LDH₅ and LDH₄/LDH₅ ratio, mainly due to the higher LDH₅ activity observed. In addition to the absolute amount of PMNs, the LDH₄/LDH₅ ratio appeared to be a good discriminator between the infectious and noninfectious group.

Distinguishing ARDS from active pulmonary infections at an early stage is of great clinical importance as both entities require a different therapeutic approach [31]. Meduri et al. [32] and Steinberg et al. [33] reported a marked neutrophilia in BALF in ARDS, predominantly in early ARDS. These studies included patients with sepsis-induced ARDS. The noninfectious group of the present study included only four patients suffering from ARDS. Compared to the infectious group, these four cases had a lower number of PMNs, lower LDH₅ activity and a higher LDH₄/LDH₅ ratio. These results should be interpreted with care due to the rather limited sample size of the studied ARDS population and the different time between the onset of ARDS and lavage. The results of
B(ALF depend on the phase of ARDS (exudative, proliferative and fibrotic) [34]. Furthermore, different mechanisms may be present in ARDS that develop after trauma, infectious aetiology such as sepsis, or other conditions. Future studies are required to evaluate the clinical relevance of including cellular and biochemical analysis to distinguish patients with ARDS from patients with pneumonia.

Alkaline phosphatase in bronchoalveolar lavage fluid

Type II pneumocytes are important in the repair of alveolar epithelium after injury and response to oxidant stress (such as hypoxaemia). Normally, type II cells are not present in BALF [9, 35]. The ALP activity, a marker of type II cell damage and/or proliferation was reported to be increased in BALF after exposure to pneumotoxicants [9, 24, 36] and associated with progression of fibrosis [35]. In both patient populations of the present study, the BALF samples showed high ALP activity compared to the control group, indicating type II involvement in the pathophysiologic process. However, no difference in ALP activity was found between BALF samples of infectious and noninfectious aetiology, respectively. As a result, ALP activity, in contrast to LDH, did not differentiate between inflammatory processes of infectious or noninfectious aetiology.

Advantage of additional enzyme detection in bronchoalveolar lavage fluid

The sensitivity of detecting an increase of the enzyme activity in BALF was found to be minimally dependent on the volume of fluid used for lavage (data not shown) in contrast to cell counting [37]. Furthermore, assessing enzymatic markers of inflammation and cell damage, such as ALP, LDH and LDH isoenzyme activities, appeared to be of additional value to identify which inflammatory cells were involved in the pathologic process. Therefore, if it is not possible to assess the total and differential cell count, monitoring biochemical changes may be of value to establish the inflammatory cell status of a patient. Moreover, due to interobserver differences, cell counting can vary [38], whilst the measurement of enzymes is well standarized and reproducible. Measurement of LDH, ALP activity and LDH isoenzymes can be achieved within a 2-h period. Thus, enzyme activities can be available without delay. More important, these relatively cheap and easy to perform measurements are applicable in every hospital.

Conclusion

In conclusion, assessment of the total and differential cell count as well as monitoring biochemical changes in bronchoalveolar lavage fluid appeared to be of additional value in the diagnostic work-up of patients with suspected pneumonia. In particular, the absolute number of polymorphonuclear neutrophils, but even more the lactate dehydrogenase/lactate dehydrogenase 5 ratio in bronchoalveolar lavage fluid demonstrated a positive predictive value in distinguishing disorders of infectious and noninfectious aetiology. Further studies are required to support the potential clinical relevance of adding cell counting and biochemical analysis to the microbiological analysis of bronchoalveolar lavage fluid in general.

References