Are pulmonary haemostasis and fibrinolysis out of balance in equine chronic pneumopathies?

Ann Kristin Barton1,*, Caroline Wirth1, Angelika Bondzio2, Ralf Einspanier2, Heidrun Gehlen1

1Equine Clinic, and 2Institute of Veterinary Biochemistry, Freie Universitaet Berlin, 14163 Berlin, Germany

*Corresponding author: Tel: +49-177-7879733; Fax: +49-30-838-62529; E-mail: Ann-Kristin.Barton@fu-berlin.de
Results of clinical examination, bronchoalveolar lavage fluid (BALF) cytology, acute phase proteins and markers of pulmonary haemostasis and fibrinolysis (fibrinogen, serum amyloid A, D-dimers) were compared between controls and horses affected by respiratory disease. Using a clinical scoring system, 58 horses were classified as free of respiratory disease (controls, n=15), recurrent airway obstruction (RAO, n=18), inflammatory airway disease (IAD, n=14) or chronic interstitial pneumopathy (CIP, n=11). No significant differences were found in fibrinogen concentration, but descriptive data analysis showed a trend towards lower values for controls (median 0.0024 g/l) compared to chronic pneumopathies (median 0.0052 g/l), in particular RAO (median 0.0062 g/l). Fibrinogen concentration was positively correlated with the percentage of neutrophils in BALF cytology ($r_s=0.377$, $p=0.004$). SAA concentrations were very low and in 65.5% of samples below the limit of detection. D-dimer concentrations were also very low and quantifiable concentrations could only be measured after ultrafiltration and only in RAO (median 0.1 mg/l). In conclusion, there was only slight evidence of increased coagulatory activity in equine chronic pneumopathies apart from RAO. It remains speculative, if fibrinogen and D-dimers are increased due to their role as acute-phase proteins or if the data may be interpreted as a misbalance of coagulation and fibrinolysis.

**Keywords:** chronic interstitial pneumopathy, horse, inflammatory airway disease, inflammatory marker, recurrent airway obstruction,
Introduction

While numerous studies have demonstrated a misbalance of pulmonary haemostasis and fibrinolysis in various forms of pulmonary disease in men, little data is available for the horse. Respiratory disease, in particular recurrent airway obstruction (RAO) and inflammatory airway disease (IAD), affects a large number of racing and performance horses, may cause exercise insufficiency and therefore has a high impact on the career of sports horses. In chronic pulmonary disease, remodeling results in decreasing airway lumen, increased smooth muscle mass, peribronchial fibrosis, epithelial cell hyperplasia and impaired airway function [24, 27]. Fibrosis formation is the ultimate result of a misbalance between pulmonary haemostasis and fibrinolysis, either caused by procoagulatory factors favoring hemostasis, downregulation of fibrinolysis or a combination of both. Regulation of remodeling may be a key for developing new therapeutics and disease management [26].

Early studies [17, 18] found that the cell-free supernatant of respiratory secretions from horses affected by RAO may induce coagulation of plasma. This pro-coagulatory activity was correlated with the severity of respiratory disease and the percentage of neutrophils in tracheobronchial aspirate (TBA) and bronchoalveolar lavage fluid (BALF) cytology. As shown for various forms of pulmonary disease in men [19, 22, 39], this pro-coagulatory state is caused by increased tissue factor-factor VII complex activity in the horse [17, 18] and therefore the extrinsic pathway of coagulation.

Fibrin and its derivation products may play a central role in equine pulmonary disease. Increases in soluble fibrinogen derivates (SFAs) were found in the majority of cell-free supernatants of respiratory secretions from horses suffering from chronic pneumopathies, again correlating with neutrophil percentages and severity of disease [45]. Using immune histochemistry, fibrin and fibrinogen were found in tracheobronchial aspirates and histologic preparations of thickened alveolar septae and bronchial mucus accumulations. In addition to
cases of RAO this was even more the case for chronic broncho-interstitial and granulomatous interstitial pneumopathies [45].

Although not directly coagulation- and fibrinolysis-associated, serum amyloid A (SAA) was also evaluated as part of this study, as fibrinogen is not only a product of coagulation, but also an acute-phase protein, so we decided to evaluate another parameter known for its sensitivity for inflammatory processes [23, 25, 34]. SAA has been shown to be involved in pulmonary inflammation in men, where increased concentrations were found in asthma and chronic obstructive pulmonary disease (COPD) [1, 2, 30, 32]. Lavoie-Lamoureux et al. [25] also found increased concentrations in plasma of horses affected by RAO. To our knowledge, SAA has not been evaluated in equine BALF.

So far, no studies have been published focusing on equine pulmonary fibrinolysis, but some information is available from other organ systems. In colic, sepsis, septic and non-septic arthritis evidence exists not only for alterations in coagulation, but also for changes in fibrinolysis as known from human medicine [3, 8, 29, 36]. The affected parameters may be measured systemically in plasma, but local changes in fluids from affected body cavities have been found to be even more pronounced, like in peritoneal fluid from horses with colic or synovia in septic and non-septic arthritis [9, 11, 35]. Therefore, we used BALF in this study to compare markers of haemostasis and fibrinolysis in different forms of equine chronic pneumopathies.

In dogs, pulmonary fibrinolysis has been studied in context with micro thromboembolism caused by heartworm infection. D-dimer deposits in lungs and kidneys and increased plasma values of D-dimers were associated with pulmonary thromboembolism and microfilaremic status [5].

A study in cats investigated the effects on lungs of inhibiting the the fibrinolytic system using tranexamic acid (TXA). The inhibition of fibrinolytic system appeared to have caused
emphysematous alterations, alveolar wall destruction and collagen accumulation possibly by causing microthrombosis leading to mechanical blockage-ischemic changes, or by causing secondary fibrinolysis as a result of fibrin degradation products affecting local plasminogen activators and proteases. An injury-repair process also appeared to have occurred [20].

Pulmonary haemostasis and fibrinolysis has been studied extensively in mice, as mouse-models have been used for research focusing on human asthma and COPD. Proteins of the haemostasis and fibrinolysis systems were shown to be involved in the pathomechanisms of hypersensitivity of the airways. After inhalation of an aerosol containing fibrinogen followed by thrombin mice were found to be hypersensitive in a metacholine provocation test compared to animals after inhalation of saline [44]. The presence of thrombin, fibrinogen and fibrin in the airways of asthmatic subjects indicates inhibition of fibrinolysis as well as increased haemostatic activity [33; 44]. Yuda et al. [46] found increased thrombin and soluble tissue factor in BALF of exacerbated mice after ovalbumin challenge. Treatment with anticoagulatory APC inhibited the Th-2 mediated immune response and obstruction of the airways.

Coagulation factor Xa plays a central role in pulmonary remodelling. Increased concentrations of Factor X mRNA were found in pulmonary tissue and BALF of mice after allergen challenge, while the use of a factor X inhibitor led to decreased respiratory mucosa thickness and collagen deposition in the pulmonary tissue. A factor Xa dependant mucin production was also found [40].

The aim of this study was to compare clinical and cytological data with fibrinogen, SAA, PAI-1 and D-dimer concentrations between healthy individuals and different of groups of chronic pneumopathy including RAO, inflammatory airway disease (IAD) and chronic interstitial pneumopathy (CIP) to evaluate a possible mismatch between the two branches of hemostasis, coagulation and fibrinolysis.
Materials and Methods

Preparticipation examination

Horses were examined for 3 successive days from the day of admission until discharge. A total of 61 horses (height 154 ± 12 cm, age 12 ± 5 years, BDW 469 ± 96kg) were examined, of which 15 had no clinical signs or history of respiratory disease and 46 were presented to the Equine Clinic of the Free University of Berlin with a history of chronic lower airway disease. Sampling of horses affected by respiratory disease was not classified as animal experiments by the State Office of Health and Social Affairs Berlin (LaGeSo), sampling of control horses was approved (reference nr. L0294/13). The owners gave permission to involve their horses in the study.

A pre-participation examination was performed including clinical examinations of the respiratory tract, blood gas analysis (BGA), endoscopy and cytology of BALF using a clinical scoring system recommended by an international workshop [15, 31, 37]. This scoring system was modified by including neutrophil percentages in BALF instead of those in tracheobronchial aspirates (table 1).

In detail, groups were defined as follows: (1) Controls, no history of respiratory disease, clinical score < 2, no tracheal secretions, low cellular density and neutrophils ≤ 10% in BALF cytology, AaDO₂ ≤ 7mmHg, exclusion of acute signs of infection (leukocytosis, fever, depression). (2) RAO, history of recurrent cough or dyspnea, clinical score > 6, high amount or viscosity of tracheal secretions, high cellular density and neutrophils ≥ 25% in BALF cytology, AaDO₂ > 7 mmHg, exclusion of acute signs of infection (leukocytosis, fever, depression) according to Robinson [37]. (3) IAD, history of cough or exercise insufficiency, clinical score 2-6, low to moderate amount or viscosity of tracheal secretions, increased cellular density and neutrophils ≥ 10% or mast cells ≥ 2% or eosinophils ≥ 0.1% in BALF cytology, AaDO₂ > 7 mmHg, exclusion of acute signs of infection (leukocytosis, fever,
depression) according to Couëtil et al. [10]. (4) CIP, history of exercise insufficiency, clinical
score 2-6, low to moderate amount or viscosity of tracheal secretions, increased cellular
density and ratio of macrophages : neutrophils ≥ 2.6 : 1 in BALF cytology, increased
interstitial opacity of thoracic radiographs, exclusion of acute signs of infection (leukocytosis,
fever, depression) according to a review on equine CIP by Dieckmann et al. [12].

**BALF collection and processing**

Following endoscopy, 20 ml of 2% lidocaine® (bela-Pharm GmbH, Vechta, Germany) were
infused around the carina of the trachea. The bronchoalveolar lavage was performed using a
300 cm Silicone Bronchoalveolar Lavage Catheter (Smiths Medical ASD, Inc, USA), which
was passed nasally into the distal respiratory tract and wedged into the bronchus by mean of
an air balloon. Five hundred milliliters of pre-warmed Phosphate Buffered Saline® (Lonza,
Verviers, Belgium) were infused into the bronchi through a BAL catheter as recommended by
the International Workshop on Equine Chronic Airway disease [37] and immediately
aspirated.

After the sample was collected, the recovered volume was recorded. The collected volume
was divided into 2 portions, one for cytological examination and the second for biochemistry.
For cytological examination, the samples were centrifuged at 1500 rpm for 10 min, then a
direct smear was air-dried, stained using the May-Grünwald Giemsa method (Sigma-Aldrich
Chemie GmbH, Germany) and 500 cells were examined under a 1000x magnification (oil
immersion). For biochemical analysis, BALF were centrifuged in the “Table Top Refrigerated
Centrifuge Hermle Z326K” (Hermle Labortechnik GmbH, Germany) at 1500 rpm for 10 min
and the temperature was 4°C. The cell-free supernatant was collected and stored at -80°C
until assayed. Samples were transported to external laboratories on dry-ice.

**Group classification**

According to the results of the pre-participation examination, the 61 horses presented for
participation in this study were classified as follows: 15 horses (24.5%) were classified as free
of respiratory disease (controls), 18 (29.5%) as RAO, 14 (23.0%) as IAD, 11 (18.0%) as CIP
and 3 horses (5.0%) suffered from acute respiratory infection, which were excluded from the
study. In the remaining 58 horses, insignificant differences between disease groups were
found in the severity of dyspnea, amount and viscosity of tracheal secretions, PaO\textsubscript{2} and
AaDO\textsubscript{2}. The overall results of the clinical examinations are presented in Table 2. Percentages
of macrophages, lymphocytes, mast cells and in particular neutrophils were also highly
significant different between controls and different disease groups (Table 3).

**Evaluation of fibrinogen**

Fibrinogen concentrations were measured in samples from 58 horses using automatized
immune turbidimetry (C701 module, Cobas® 8000 series, Roche Diagnostics Germany) in
BALF supernatant. The specific limit of detection was 0.001 g/l.

**Evaluation of SAA**

SAA was evaluated in samples from 58 horses using a sandwich-ELISA assay („Phase”™
Serum Amyloid A Assay (SAA) – Multispecies, Tridelta Development Ltd., Ireland). All
BALF supernatant samples were tested undiluted; the limit of detection was 0.01 µg/ml.

**Evaluation of D-dimers**

D-dimer concentrations in BALF supernatant were measured in samples from 58 horses using
an automatized analyser (Sysmex® CA-1500 system, Sysmex Corporation, Germany) by
immune turbidimetry (INNOVANCE® D-dimer test, Siemens Healthcare Diagnostics,
Germany). The limit of detection was 0.19 mg/L FEU (fibrinogen equivalent units). As most
samples were below this limit of detection, 18 samples of controls and RAO were
concentrated by ultrafiltration (2000g for 60 minutes, Rotina 420R, Andreas Hettich GmbH &
Co. KG, Germany).

**Evaluation of PAI-1**
For PAI-1 measurements three ELISA assays of different manufacturers were used: a competitive and a sandwich ELISA with equine antibodies (Horse Plasminogen activator inhibitor 1 ELISA Kit, Cusabio Biotech Co., Ltd and Nori™ Equine Serpin E1/PAI-1 ELISA, Genorise Scientific, Inc.) and a humane assay (Quantikine® ELISA, Human Serpin E1/PAI-1, R&D Systems® Inc.) were tested for suitability for measuring PAI-1 in equine BALF.

Statistical analysis

Data were statistically analyzed and graphically presented using SPSS Statistics® (Version 22.0 SPSS Campus Edition, SPSS Inc., USA). The data were tested for normal distribution using the Kolmogorov-Smirnov and Shapiro Wilks test and expressed as mean ± standard deviation (SD) for normally distributed and as median and 25/75% quartiles for non-normally distributed data. The level of significance was set at $P < 0.05$.

Kruskal Wallis H test was used to compare between healthy horses group and different disease groups followed by Post-Hoc testing using the Dunett test for Post Hoc Analysis.

Results

Assays for quantification of fibrinogen, SAA, D-dimer and PAI-1 concentrations were performed in BALF supernatant from 58 horses. In detail, the results were as follows:

Fibrinogen

Concentrations of samples below the limit of detection at 0.001g/l were defined as 0.0005g/l for statistical analysis. Although no significant differences were found between disease groups, descriptive data analysis showed a trend towards lower values for controls (median 0.0024g/l) compared to all chronic pneumopathies (RAO, IAD and CIP, median 0.0052g/l) and in particular in RAO (median 0.0062g/l) as shown in figure 1. 40% of controls’ samples were below the limit of detection, while this was the case for only 16% of chronic pneumopathies, namely 5.6, 21.4 and 27.7% of RAO, IAD and CIP samples, respectively.
The fibrinogen concentration was positively correlated with the percentage of neutrophils in BALF cytology ($r_s=0.377$, $p=0.004$) as shown in figure 2.

**SAA**

Concentrations of samples below the limit of detection at 0.01 μg/ml were defined as 0.005 μg/ml for statistical analysis. Overall, concentrations of SAA were very low and in the majority of samples (65.5%) below the limit of detection. No significant differences were found between controls and chronic pneumopathies in general or the different disease groups.

**D-dimers**

Immune turbidimetric analysis of D-dimers revealed concentrations below the test specific limit of detection in 94.8% of samples. The only samples, in which concentrations could be quantified, belonged to the RAO group. Therefore, samples of this group and five controls’ samples were subjected to ultrafiltration, which yielded quantifiable concentrations in all but one RAO samples, while the controls’ samples remained below the limit of detection. The median concentration in RAO was 0.1 (0.07/0.185) mg/l with a range of 0.035-0.33 mg/l.

**PAI-1**

PAI-1 concentrations were measured using three sorts of ELISA assays by three different manufacturers, but unfortunately, all assays did not produce reliable standard curves or quantifiable results in neither plasma nor BALF samples. Therefore, no results were available for statistical analysis.

**Discussion**

The aim of this study was to characterize the fibrinolytic activity in equine BALF in different chronic pneumopathies. Specific activators of fibrinolysis are t-PA and u-PA, while the system is inhibited by plasminogen inhibitors (PAI-1 and -2), α2-antiplasmin and the degradation products of fibrinolysis, the D-dimers. Several factors need to be evaluated to
find out, whether the system is ultimately activated or inhibited. For example, increased D-
dimer concentrations might lead to the suspicion of increased fibrinolytic activity, but they
may also occur after severe coagulation, massive fibrin production and increased fibrinolysis,
but still being insufficient to counteract coagulation, leading to a misbalance towards
hypercoagulation in the end. Therefore it is essential to evaluate specific regulators of the
fibrinolytic system as well. PAI-1 was chosen as the parameter of interest in this study, as
pulmonary fibrinolysis in other species is strongly dependent on u-PA and PAI-1 [7, 21, 38].
PAI-1 concentrations were measured using three sorts of ELISA assays by different
manufacturers, but unfortunately, none of the available assays was found feasible to produce
reliable standard curves or quantifiable results in neither plasma nor BALF samples.
Therefore, we had to focus on fibrinogen and D-dimers and the ratio between these two. In
case of overwhelming coagulative activity, a higher ratio caused by a more severe increase in
D-dimers might be suspected, while a lower ratio would be the consequence of overwhelming
fibrinolysis. Unfortunately, even after ultrafiltration D-dimers could not be quantified in
controls, which made the calculations of such ratios impossible in all groups apart from RAO.
In the presented study, the results of fibrinogen concentrations are in agreement to early
studies, in which this parameter, an acute phase protein and a key factor in coagulation, was
found in tracheobronchial aspirates and immune histopathologic samples of horses suffering
from chronic respiratory disease [43, 45]. Fibrinogen increases may be explained by increased
permeability of the capillary endothelium and the alveolar epithelium going along with
plasma exudation and leakage of plasma proteins from the vasculature into the alveolar
lumen, which has been shown in men and is suspected in equine disease [4, 16, 43, 45]. The
positive correlation of fibrinogen concentrations to the percentage of neutrophils in BALF
cytology supports its role in the pathogenesis of RAO, in which neutrophils play a central role
as a causative agent or a consequence of different inflammatory mechanisms [14]. This is
supported by the results of Winder et al. [45] showing a correlation of fibrinogen
concentration and neutrophil percentages in tracheobronchial aspirates.

As D-dimer concentrations could only be quantified after ultrafiltration and only in samples
of horses suffering from RAO in exacerbation, this product of increased coagulation and
fibrinolysis may be produced in a considerable amount in more severe inflammatory
processes only. Obviously, it was not produced in minor stages of inflammation as in IAD or
RAO in remission, and it may not be produced anymore in chronic interstitial pneumopathies,
in which the exudative phase of inflammation is over and fibrosis formation has already
occurred. Studies in human asthma, a disease resembling many features of equine RAO, in
ARDS and pneumonia have found increased D-dimer concentrations in respiratory secretions
like sputum and BALF as well [4]. A clear correlation was found between the grade of
inflammation and D-dimer concentrations, which were in a similar range than in samples of
our study. Coagulation and fibrinolysis may even be triggered in the horse by minor
inflammatory stimuli compared to humans, as equine RAO has a more chronic nature than
ARDS or acute pneumonia, but the measured concentrations were still similar. Inflammatory
processes in other organ systems of the horse like intestinal disease and arthropathies go along
with clear increases of this product of coagulation [6, 13, 35, 36]. Studies in human and
equine arthropathies have shown that D-dimer concentrations in synovial fluid compared to
plasma were much higher in horses than in men [36]. The basal value in plasma also seems to
be higher in horses [41], therefore species specific differences might exist in this parameter,
with horses showing more pronounced increases during inflammatory processes. More
sensitive assays are necessary to evaluate the role of D-dimers in IAD, RAO in remission and
chronic interstitial pneumopathies. Ultrafiltration was not performed in these groups as all
samples were below the limit of detection, but nevertheless, it may have also yielded
quantifiable concentrations. In the end, it cannot be ruled out that D-dimers may play a more
central role in respiratory disease than could be proven by our data.

As expected, the results of our study do not support an important role of SAA in equine chronic pneumopathies. In the majority of samples, SAA was below the limit of detection, although significant increases were found in plasma in severe RAO [25]. It seems logical that plasma exudation during inflammation would also lead to leakage of SAA into the alveolar space, but there are several reasons that SAA may be below the limit of detection. First, there is the dilution effect of the BALF. Second, SAA is a parameter rising quickly, but with a very short half life. In the study of Lavoie-Lamoureux [25] high concentrations were measured in plasma 7 and 30 days after antigen challenge, which were easy to quantify. Most horses presented for our study had a history of chronic respiratory disease and most had not shown acute deterioration of clinical signs shortly before. Therefore, it can be suspected, that SAA concentrations had fallen prior to sampling.

Partly insurmountable weak points of this study were group definitions and feasibility of assays. Although IAD and RAO in remission were planned as two distinct groups, it was not possible to differentiate clearly between these two. Anamnestic information of respiratory distress was often unreliable and the majority of owners did not agree to a natural challenge test. Descriptions of equine CIP are rare in the literature and an international consensus statement is missing, so definition of this group was based on a quite old clinical case series including only 12 horses [12]. Thoracic radiography showing interstitial patterns is not very specific for CIP and may also be found in RAO and IAD [28, 42]. Again, the majority of owners did not agree to lung biopsies. We tried to face these problems by calculating correlations between parameters studied with clinical and cytological parameters over all 58 horses.

Regarding the assays, one should decide for the method of highest sensitivity, so we principally preferred ELISA systems, but due to the fact that many of these were not validated
for horses, we chose other validated or at least commonly used assays for our study. A validated ELISA assay was available for SAA and has been used in horses [25, 34], but still, we had many SAA samples below the limit of detection. For D-dimers, no ELISA was available anymore, so we had to go with immuno-turbidimetry, in which ultrafiltration was essential to yield quantifiable concentrations. Fibrinogen was also evaluated by immuno-turbidimetry, which is commonly used in horses and was offered by a commercial laboratory. The biggest problem was PAI-1, in which none of the three assays tested produced reliable results, although two species specific tests were tested as described above.

In conclusion, this is the first study, in which fibrinogen, D-dimers and SAA concentrations were evaluated in BALF of healthy horses in comparison to horses affected by different forms of chronic pneumopathies. Even if the aims of the study could only be realized in part, the results of the study allow suspecting a misbalance between coagulation and fibrinolysis in respiratory disease with a tendency towards fibrin formation. Further studies and improvements in assays are essential to further characterize pathophysiologic background, mechanisms and factors of influence.

**Conflict of Interest**

There is no conflict of interest.
References


9. Collatos C, Barton MH, Prasse KW, Moore JN. Intravascular and peritoneal


18. Gruenig G, Hulliger C, Hermann M, Winder C, von Fellenberg R. Separation of


35. Ribera T, Monreal L, Armengou L, Rios J, Prades M. Synovial fluid D-dimer...


Fig. 1. Fibrinogen concentrations over different disease groups. Although no significant differences were found, a clear trend towards lower values in controls (median 0.0024g/l) compared to RAO in exacerbation (median 0.0062g/l) is visible.

Fig. 2. Correlation of fibrinogen concentrations with percentage of neutrophils in BALF.
Table 1. Clinical score, modified from Ohnesorge et al. (1998), this score was modified by including BALF cytology instead of tracheal aspirates

<table>
<thead>
<tr>
<th></th>
<th>Score</th>
<th>Max. points</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coughing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No cough after manual compression of larynx</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Coughing during manual larynx compression</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Very frequent coughing</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Spontaneous coughing</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Prolonged expiration</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Abdominal breathing</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Sinking of the intercostal area</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>Dyspnoea at rest</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nostril flare</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Heaves line</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Anal pumping</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Heaves line</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Nostril flare</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Anal pumping</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>Lung percussion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Handbreadth</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Damping</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Rattling</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><strong>Lung auscultation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crackle</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Wheezing</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Significantly increased secretions with moderate viscosity</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Respiratory endoscopy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highly increased secretions with high viscosity</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Thickened carina of the trachea</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Neutrophils &lt; 10%</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Neutrophils 10-15%</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Neutrophils 16-25%</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Neutrophils &gt; 25%</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>BALF cytology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AaDo₂: 0-7 mmHg</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>AaDo₂: 7-14 mmHg</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>AaDo₂: &gt; 14 mmHg</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Results of clinical examinations, means ± standard deviations. The (*) shows significant differences in comparison to controls at $P < 0.05$

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=15)</th>
<th>RAO (n=18)</th>
<th>IAD (n=14)</th>
<th>CIP (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyspnea</td>
<td>0 ± 0</td>
<td>1.61 ± 1.0*</td>
<td>0.07 ± 0.27</td>
<td>0.36 ± 0.92</td>
</tr>
<tr>
<td>Lung auscultation</td>
<td>0 ± 0</td>
<td>0.35 ± 0.78</td>
<td>0.22 ± 0.65</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Endoscopy</td>
<td>0 ± 0</td>
<td>1.78 ± 0.43*</td>
<td>1.14 ± 0.66*</td>
<td>0.91 ± 0.7*</td>
</tr>
<tr>
<td>BALF cytology</td>
<td>0 ± 0</td>
<td>3 ± 0*</td>
<td>1.57 ± 1.02*</td>
<td>0.45 ± 0.69</td>
</tr>
<tr>
<td>Blood gas analysis</td>
<td>0.27 ± 0.4</td>
<td>0.78 ± 0.73*</td>
<td>0.14 ± 0.36</td>
<td>0.36 ± 0.81</td>
</tr>
<tr>
<td>Total exam score</td>
<td>0.33 ± 0.46</td>
<td>8.12 ± 2.17*</td>
<td>3.36 ± 1.27*</td>
<td>2.45 ± 2.73*</td>
</tr>
</tbody>
</table>

Table 3. BALF cytology, the results of cell percentages are expressed as medians (min/max)

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=15)</th>
<th>RAO (n=18)</th>
<th>IAD (n=14)</th>
<th>CIP (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophages [%]</td>
<td>58 (53.7/59.8)</td>
<td>17.3 (8.3/28.3)*</td>
<td>49.5 (30.7/52.5)</td>
<td>54 (40/62.8)</td>
</tr>
<tr>
<td>Lymphocytes [%]</td>
<td>37.4 (32.8/42.7)</td>
<td>17.6 (6.9/30.2)*</td>
<td>30.5 (24.4/38.5)</td>
<td>33.4 (23.2/40)</td>
</tr>
<tr>
<td>Neutrophils [%]</td>
<td>2 (1.2/4.6)</td>
<td>51.4 (45.1/84.8)*</td>
<td>16.4 (11.9/22.6)*</td>
<td>7.2 (5.8/10)</td>
</tr>
<tr>
<td>Eosinophils [%]</td>
<td>0 (0/0.2)</td>
<td>0 (0/0.5)</td>
<td>0 (0/1.3)</td>
<td>1 (0/1.8)</td>
</tr>
<tr>
<td>Mast cells [%]</td>
<td>1.6 (1.2/2.6)</td>
<td>1 (0.1/1.6)</td>
<td>3.1 (0.9/4.7)</td>
<td>3.4 (2/7.2)</td>
</tr>
</tbody>
</table>

The (*) shows significant differences in comparison to controls at $P < 0.05$