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Predictive value of BAL cell differentials in the diagnosis of interstitial lung diseases

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Predictive value of BAL cell differentials in the diagnosis of interstitial lung diseases. L. Welker, R.A. Jörres, U. Costabel, H. Magnussen. ©ERS Journals Ltd 2004.
ABSTRACT: The current authors aimed to quantify how the likelihood for a given diagnosis changes with the knowledge of bronchoalveolar lavage (BAL) cell differentials.

As an initial estimate (*a priori* probability), frequencies of final diagnoses were taken. Using categorisations for cell differentials, *a posteriori* probabilities were then derived for each disease, according to Bayes. The analysis was performed in three of five groups of diagnoses suspected prior to BAL: interstitial lung disease (ILD; n=710), inflammatory disease (n=583), or lung tumour mimicking ILD (n=455).

Overall, out of 1,971 patients, 18.3% had sarcoidosis, 7.7% usual interstitial pneumonia (UIP), 4.4% extrinsic allergic alveolitis (EAA), and 19.0% tumours. In the group with suspected ILD, the likelihood for sarcoidosis increased from 33.7 to 68.1% when lymphocyte numbers were 30–50% and granulocyte numbers were low; the likelihood for UIP increased from 15.8 to 33.3% when lymphocyte numbers were <30% with granulocytes elevated. CD4/CD8 was informative, especially in sarcoidosis and EAA. Despite considerable increases, the likelihood of rare diseases rarely reached appreciable values. Similar results were obtained in the other two groups of suspected diagnoses.

In conclusion, these data suggest that bronchoalveolar lavage cell counts *per se* provide substantial diagnostic information only in relatively frequent diseases, such as sarcoidosis and usual interstitial pneumonia, and are less helpful in infrequent diseases. *Eur Respir J 2004; 24: 1000–1006.*

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Bronchoalveolar lavage (BAL) is a standard tool in the diagnosis of lung diseases [1–3], and the analysis of differential cell counts in BAL fluid (BALF) is part of clinical routine. This is reflected in a great number of data on BALF composition in different disorders, and normal ranges of cellular composition have been assessed, as well as recommendations for its use [4–8]. A major area of interest is the diagnosis of interstitial lung disease (ILD) [9–12]. In clinical practice, a BAL result obtained in an individual patient is compared with the pattern expected for a suspected disease, and the results are then put into the diagnostic puzzle as far as possible. BAL data are, however, subject to considerable variability, and the number of potential diseases is far greater than the number of safely discernible cellular patterns. Thus, only in rare instances, the data lead to a unique conclusion; in the majority of cases, BAL cell differentials are only able to render some diagnoses more likely and to exclude others with some probability.

This uncertainty, in combination with differences in clinical setting and experience, results in different opinions about the diagnostic value of BAL among clinicians. It is unclear to which degree or under which conditions BAL cell counts add helpful information in the diagnostic work-up of either prevalent or rare lung diseases. To answer these questions, the gain in information post- *versus* pre-BAL can be determined by assessing how the likelihood of a disease changes as a function of the BAL result. If there is no change in likelihood, there is no information. To incorporate the full scope of clinical information in individual patients or differences in clinical experience seems impossible with finite data sets. Thus, the simpler approach that a few categories of suspected diagnoses are the only information available prior to BAL might be adopted. The gain in information should be revealed even under these conditions. While in previous studies [13, 14] logistic regression has been used for statistical analysis, the current authors aimed to directly assess the changes in probability in a manner close to that adopted by most clinicians in the diagnostic process.

Therefore, a retrospective analysis of BAL data from the current authors' laboratory was performed, using the approach of Bayes for quantification of *a posteriori versus a priori* probabilities. The analysis was done for different groups of diseases suspected prior to BAL, with special emphasis on ILD.

Materials and methods

Patients

Between January 1997 and November 2003, a total of 3,975 BALF samples from 3,118 patients (1,797 males, 1,321 females; mean±SD age, 56.7±14 yrs) were evaluated. A clinically and/or histologically established final diagnosis

was available in all patients. If BAL was performed more than once, the first was chosen for analysis. A total of 1,971 patients were included, all of whom were without steroid or other immunoregulatory therapy, and who had a recovery of $\geq$25 mL, viability of $\geq$75%, and $\leq$15% epithelial cells in BALF. In 1,304 patients, final diagnosis was based on the overall clinical picture, and, in 667 patients, it was additionally proven by biopsies. All available diagnostic information was used and all diagnoses were verified by experienced doctors. The samples were catagorised into five groups on the basis of the clinically suspected disease prior to the performance of BAL: ILD (n=710, 364 males, 346 females, 53±16 yrs), inflammatory disease (pneumonia; n=583, 299 males, 284 females, 55±14 yrs), lung tumour (mimicking ILD; n=455, 294 males, 161 females, 61±12 yrs), exposure to dust and fibres (n=161) and others (n=62). The first three groups (n=1,748) were chosen for analysis in the present study.

Analysis of bronchoalveolar lavage fluid

After the volume of recovered BALF had been assessed, the fluid was filtered through a layer of sterile gauze, centrifuged (15 min, 4°C, 65×*g*) and resuspended. Total cell counts were assessed in a Neubauer chamber and viability was determined by trypan blue exclusion. A cytospin slide was prepared from 50,000 cells (600 cpm, 15 min; Heraeus Sepatech Omnifuge 2.0 RS; Heraeus Sepatech, Hanau, Germany), stained with May-Grünwald-Giemsa and used for the cytological examination of $\geq$500 cells. For immunocytological analysis, up to four aliquots were incubated with fluorescein isothiocyanate-labelled monoclonal antibodies (Dako, Hamburg, Germany) against CD1 (Langerhans cells), CD3, CD4 and CD8 (30 min, 4°C). After washing, cytospin slides were prepared, and on each slide $\geq$100 lymphocytes were counted to determine the percentages of CD1-, CD3-, CD4- and CD8-positive cells, as well as the CD4/CD8 ratio.

Data analysis

The relative frequencies of final diagnoses, as based on all available information, were taken as estimates of *a priori* probabilities. The current authors then computed *a posteriori* probabilities according to Bayes' rule [15] for each disease, using categories for cell differentials and the CD4/CD8 ratio that were similar to previous studies [12, 16]. The analysis was performed in each of the three groups of suspected diseases in which sufficient data were available (ILD, inflammatory disease, lung tumour), but, owing to space reasons, only values for the ILD group are given in detail. *A priori* and *a posteriori* probabilities were statistically compared as proportions. No correction for multiple testing was made, as no rational choice for multiplicity seemed possible; instead, comparisons showing $p<0.001$ were marked separately in the tables. In addition, for BAL data of the ILD group, mean values and standard deviations were computed. These data were compared between final diagnoses by one-way ANOVA and Newman-Keuls *post hoc* tests or Mann-Whitney U-tests. In all analyses, statistical significance was assumed for $p<0.05$.

Results

Summary of patients' final diagnoses

In 583 (33.4%) of the 1,748 patients, the final diagnosis was inflammation, such as pneumonia, bronchial asthma, chronic obstructive pulmonary disease, tuberculosis and/or pleuritis. A benign or malignant tumour was found in 455 patients (26.0%). A total of 685 patients (34.6%) were diagnosed as having one of four major ILDs: sarcoidosis, usual interstitial pneumonia (UIP), extrinsic allergic alveolitis (EAA), or nonspecific interstitial pneumonia (NSIP). Sarcoidosis (18.3%) was most frequent among these, followed by UIP (7.7%), NSIP (4.4%) and EAA (4.4%). In 246 patients (12.4%), more rare ILDs, such as bronchiolitis obliterans organising pneumonia (BOOP), connective tissue disease (collagenosis), histiocytosis X, chronic eosinophilic pneumonia (CEP), lymphocytic interstitial pneumonia, desquamative interstitial pneumonia, respiratory bronchiolitis-associated interstitial lung disease (RBILD), asbestosis, silicosis, Wegener's granulomatosis and alveolar proteinosis, were diagnosed. In five out of 15 patients with histiocytosis X, CD1 values were >5%, and, in three patients, were between 1 and 5%; correspondingly, seven patients showed CD1 values <1%. Overall, 96 patients (4.9%) turned out to have no lung disease. For the group with suspected ILD, *a priori* probabilities are given in table 1. These values served as a reference for assessing the changes in likelihood according to BAL results.

Bronchoalveolar lavage fluid differential cell counts

Table 2 shows the cell differentials according to final diagnoses in the group with suspected ILD, as well as data for known smokers. The percentages of lymphocytes, neutrophils and eosinophils, as well as the CD4/CD8 ratio, were significantly different between diagnoses (all $p<0.001$).

Table 1. – Numbers and probabilities (*a priori* probability) of final diagnoses in the group with suspected interstitial lung disease (ILD)

Diagnosis	All patients	Smokers
Sarcoidosis	239 (33.7)	19 (19.6)
UIP	112 (15.8)	16 (16.5)
EAA	66 (9.3)	4 (4.1)
NSIP	46 (6.5)	7 (7.2)
Tumour	29 (4.1)	11 (11.3)
Others	25 (3.5)	4 (4.1)
No fibrosis	23 (3.2)	2 (2.1)
Connective tissue disease	18 (2.5)	1 (1.0)
BOOP	17 (2.4)	
Histiocytosis X	15 (2.1)	9 (9.3)
Alveolar haemorrhage	8 (1.1)	1 (1.0)
Tuberculosis	7 (1.0)	1 (1.0)
Pneumoconiosis	7 (1.0)	2 (2.1)
Drug-induced alveolitis	5 (0.7)	
CEP	4 (0.6)	2 (2.1)
RBILD	4 (0.6)	1 (1.0)
Wegener's granulomatosis	3 (0.4)	
LIP	3 (0.4)	
Pneumocystis carinii	1 (0.1)	1 (1.0)
Alveolar proteinosis	1 (0.1)	
DIP	1 (0.1)	
Inflammatory disease	76 (10.7)	15 (16.4)

Data are presented as n (%). Only smokers with reliable information on current smoking at the time of diagnosis were analysed separately. UIP: usual interstitial pneumonia; EAA: extrinsic allergic alveolitis; NSIP: nonspecific interstitial pneumonia; others: *e.g.* obstructive sleep apnoea and cardiac failure; no fibrosis: neither ILD nor inflammatory disease nor tumour (*e.g.* persistent cough of unknown origin, suspected aspiration, reflux); BOOP: bronchiolitis obliterans organising pneumonia; CEP: chronic eosinophilic pneumonia; RBILD: respiratory bronchiolitis-associated interstitial lung disease; LIP: lymphocytic interstitial pneumonia; DIP: desquamative interstitial pneumonia.

Table 2. – Bronchoalveolar lavage differential cell counts in the most frequent diseases of the group with suspected interstitial lung disease

Final diagnosis	Subjects n	Lymphocytes***	CD4/CD8***	Neutrophils***	Eosinophils***	Total cell count
All patients						
Sarcoidosis	239	27.0 (17.0–41.0)	3.6 (2.3–6.1)	1.0 (0.0–3.0)	0.0 (0.0–1.0)	2.9 (1.6–5.0)
EAA	66	48.0 (36.0–60.0)	1.7 (0.9–3.8)	3.0 (1.0–11.0)	0.0 (0.0–2.0)	5.6 (2.9–9.1)
UIP	112	11.0 (6.0–21.5)	1.4 (0.7–2.8)	6.0 (3.0–11.0)	2.0 (1.0–6.0)	3.4 (2.0–5.8)
NSIP	46	13.5 (5.0–35.0)	1.3 (0.5–3.3)	4.0 (1.0–9.5)	1.0 (0.0–4.0)	4.9 (1.8–7.5)
BOOP	17	22.0 (10.0–29.0)	0.7 (0.4–1.1)	2.0 (1.0–3.0)	2.0 (0.0–3.0)	3.2 (1.7–6.4)
Smokers						
Sarcoidosis	19	25.0 (7.0–32.0)	5.1 (2.4–10.0)	1.0 (0.0–2.0)	0.0 (0.0–2.0)	4.9 (2.6–9.0)
EAA	4	57.0 (48.0–67.0)	0.8 (0.6–1.4)	3.0 (2.5–5.0)	1.0 (0.0–2.0)	6.0 (4.4–9.8)
UIP	16	6.0 (3.5–20.5)	1.1 (0.6–3.3)	10.5 (3.5–40.0)	3.0 (1.0–6.5)	3.8 (1.8–5.7)
NSIP	7	5.0 (1.0–7.0)	ND	2.5 (1.0–9.0)	4.0 (1.0–12.0)	4.0 (0.8–6.9)

Data are presented as median (interquartile range), unless otherwise stated. EAA: extrinsic allergic alveolitis; UIP: usual interstitial pneumonia; NSIP: nonspecific interstitial pneumonia; BOOP: bronchiolitis obliterans organising pneumonia; ND: not determined. ***: $p<0.001$, statistically significant differences between groups (all patients; ANOVA).

Specifically, lymphocytes differed between UIP, EAA, and the set of the three other diseases, CD4/CD8 between sarcoidosis and the four other diseases, neutrophils between sarcoidosis and NSIP or UIP, and eosinophils between sarcoidosis and NSIP or UIP (all $p<0.05$).

Post hoc *analysis according to Bayes*

As a first step, single variables were used to compute *a posteriori* probabilities. Tables 3–5 show the results in the patients with suspected ILD. The likelihood for sarcoidosis decreased with increasing percentage of eosinophils or neutrophils and rose with increasing CD4/CD8 ratio; the opposite was true for UIP. The likelihood for EAA was increased three-fold by a CD4/CD8 ratio <0.5, and that for CEP was increased about 50-fold, although still only 25%, when the percentage of eosinophils was >20%.

In a second step, two variables were combined (tables 6 and 7). The likelihood for sarcoidosis was raised up to twofold when the percentage of lymphocytes was elevated but not extremely high, in combination with a low percentage of granulocytes (table 6). This pattern reduced the likelihood for UIP markedly. Conversely, the likelihood for EAA and NSIP was markedly raised when the percentages of both lymphocytes and granulocytes were high. When lymphocytes were combined with CD4/CD8, the probability of sarcoidosis was doubled if the CD4/CD8 ratio was high (table 7). Conversely, NSIP and EAA gained in likelihood with a low CD4/CD8 ratio, especially when the percentage of lymphocytes was high. Likelihood measures were also computed in the subgroup of confirmed smokers (n=97), results being in parallel to those of the total group (tables 8 and 9).

As a next step, lymphocytes, granulocytes and CD4/CD8 were combined. A low percentage of lymphocytes and granulocytes and an elevated CD4/CD8 ratio raised the likelihood for sarcoidosis to >85% (table 10). When the percentage of granulocytes was high and the CD4/CD8 ratio elevated, the probability of sarcoidosis still increased more

Table 3. – Probability of interstitial lung disease (ILD) as a function of eosinophils in suspected ILD

Eosinophils %	Subjects n	*A priori*	*A posteriori*			
			<2	2–10	10–20	>20
Sarcoidosis	239	33.7	37.8	13.0***	0.0*	10.0
UIP	112	15.8	11.8*	40.3***	42.1*	20.0
EAA	66	9.3	9.3	10.4	10.5	0.0
NSIP	46	6.5	5.5	11.7	10.5	20.0
CEP	4	0.6	0.0	1.3	0.0	30.0
Others	243	34.2	35.8	23.4	36.8	20.0
Total n	710		604	77	19	10

Data are presented as %, unless otherwise stated. UIP: usual interstitial pneumonia; EAA: extrinsic allergic alveolitis; NSIP: nonspecific interstitial pneumonia; CEP: chronic eosinophilic pneumonia. *: $p<0.05$; ***: $p<0.001$ *versus* the respective *a priori* value.

Table 4. – Probability of interstitial lung disease (ILD) as a function of neutrophils in suspected ILD

Neutrophils %	Subjects n	*A priori*	*A posteriori*			
			<4	4–20	21–50	>50
Sarcoidosis	239	33.7	42.4***	19.6***	19.5	19.2
UIP	112	15.8	7.3***	31.4***	22.0	26.9
EAA	66	9.3	9.1	10.3	12.2	0.0
NSIP	46	6.5	5.2	7.4	12.2	11.5
Inflammatory disease	76	10.7	7.7	13.2	12.2	38.5***
Others	171	24.1	28.3	18.1	22.0	3.9*
Total n	710		439	204	41	26

Data are presented as %, unless otherwise stated. UIP: usual interstitial pneumonia; EAA: extrinsic allergic alveolitis; NSIP: nonspecific interstitial pneumonia. *: $p<0.05$; ***: $p<0.001$ *versus* the respective *a priori* value.

Table 5. – Probability of interstitial lung disease (ILD) as a function of CD4/CD8 in suspected ILD

CD4/CD8 ratio	Subjects n	*A priori*	*A posteriori*			
			No value[#]	<0.5	0.5–3.5	>3.5
Sarcoidosis	239	33.7	16.3***	9.1*	40.3	69.1***
UIP	112	15.8	22.7*	13.6	12.2	5.2*
EAA	66	9.3	1.5***	27.3*	17.2*	12.5
NSIP	46	6.5	7.6	18.2	5.4	3.7
Connective tissue disease	18	2.5	2.7	0.0	3.2	1.5
Others	229	32.2	49.2***	31.8	21.7*	8.1***
Total n	710		331	22	221	136

Data are presented as %, unless otherwise stated. UIP: usual interstitial pneumonia; EAA: extrinsic allergic alveolitis; NSIP: nonspecific interstitial pneumonia. *: $p<0.05$; ***: $p<0.001$ *versus* the respective *a priori* value; [#]: not measurable due to low lymphocyte numbers.

Table 6. – Probability of interstitial lung disease (ILD) as a function of lymphocytes and granulocytes in the group with suspected ILD

Lymphocytes and granulocytes[#] %	Subjects n	*A priori*	*A posteriori*					
			<30		30–50		>50	
			Low	High	Low	High	Low	High
Sarcoidosis	239	33.7	37.0	16.1***	68.1***	33.3	43.3	18.8
UIP	112	15.8	8.8*	33.3***	2.1***	9.5	1.7*	0.0
EAA	66	9.3	1.2***	3.2*	11.7	35.7***	35.0***	50.0***
NSIP	46	6.5	5.2	8.4	2.1	9.5	6.7	12.5
Connective tissue disease	18	2.5	2.0	2.8	1.1	2.4	6.7	0.0
Others	229	32.3	45.8***	36.1	14.9***	9.5*	6.7*	18.8
Total n	710		249	249	94	42	60	16

Data are presented as %, unless otherwise stated. UIP: usual interstitial pneumonia; EAA: extrinsic allergic alveolitis; NSIP: nonspecific interstitial pneumonia. *: $p<0.05$; ***: $p<0.001$ *versus* the respective *a priori* value. [#]: low (eosinophils <2% and neutrophils <4%).

Table 7. – Probability of interstitial lung disease (ILD) as a function of lymphocytes and CD4/CD8 in the group with suspected ILD

Lymphocytes % and CD4/CD8 ratio[#]	Subjects n	*A priori*	*A posteriori*					
			<30		30–50		>50	
			Low	High	Low	High	Low	High
Sarcoidosis	239	33.7	21.0***	72.2***	45.8*	75.5***	29.8	51.7*
UIP	112	15.8	22.3*	11.1	6.0*	1.9*	2.1*	0.0
EAA	66	9.3	2.3***	1.9	22.9*	13.2	42.6***	31.0*
NSIP	46	6.5	7.0	5.6	6.0	1.9	10.6	3.5
Connective tissue disease	18	2.5	2.7	0.0	2.4	0.0	4.3	6.9
Others	229	32.3	44.8***	9.3***	16.9*	7.6*	10.6*	6.9*
Total n	710		444	54	83	53	47	29

Data are presented as %, unless otherwise stated. UIP: usual interstitial pneumonia; EAA: extrinsic allergic alveolitis; NSIP: nonspecific interstitial pneumonia. *: $p<0.05$; ***: $p<0.001$ *versus* the respective *a priori* value. [#]: low (CD4/CD8 <3.5).

Table 8. – Probability of interstitial lung disease (ILD) as a function of lymphocytes and granulocytes in the group with suspected ILD (smokers only)

Lymphocytes and granulocytes[#] %	Subjects n	*A priori*	*A posteriori*					
			<30		30–50		>50	
			Low	High	Low	High	Low	High
Sarcoidosis	19	19.6	22.7	7.3	66.7*	50.0	33.3	0.0
UIP	16	16.5	4.6	31.7*	16.7	0.0	0.0	0.0
EAA	4	4.1	0.0	0.0	16.7	50.0*	66.7***	0.0
Others	58	59.7	72.8	61.0	0.0*	0.0	0.0*	100.0
Total n	97		44	41	6	2	3	1

Data are presented as %, unless otherwise stated. UIP: usual interstitial pneumonia; EAA: extrinsic allergic alveolitis. *: $p<0.05$; ***: $p<0.001$ *versus* the respective *a priori* value. [#]: low (eosinophils <2% and neutrophils <4%).

than two-fold (table 11). The likelihood for EAA rose four-fold independently of the CD4/CD8 ratio when the percentage of lymphocytes was extremely high and granulocytes were either low (table 10) or high (table 11). In contrast, the likelihood for UIP never reached appreciable values in the presence (table 11) or absence (table 10) of elevated percentages of granulocytes. Due to the small numbers within other categories, no further trivariate analyses were carried out.

To assess whether these results were specific for the group of patients with suspected ILD, the groups with suspected inflammatory disease or suspected lung tumour mimicking ILD were evaluated in an analogous way to table 6. As a result of the different distributions of diagnoses, absolute values, as well as factors of change of likelihood, were different. Despite this, the patterns of changes for sarcoidosis, UIP and EAA were similar to those found in the group with suspected ILD. In the group with suspected inflammatory disease, 48.9% of the patients turned out to have such a disease. The likelihood for sarcoidosis increased from 13.7% *a priori* to 51.2% *a posteriori* ($p<0.001$) when lymphocytes ranged 30–50% and granulocyte numbers were low (table 6). The likelihood for EAA rose from 3.1% to 25.0 or 37.5% with either low or high granulocyte numbers, and lymphocytes >50% (all $p<0.001$). In the group with suspected lung tumours, 55.8% of patients were finally diagnosed as having a tumour. The likelihood for sarcoidosis was raised from

Table 9. – Probability of interstitial lung disease (ILD) as a function of lymphocytes and CD4/CD8 in the group with suspected ILD (smokers only)

Lymphocytes % and CD4/CD8 ratio[#]	Subjects n	*A priori*	*A posteriori*					
			<30		30–50		>50	
			Low	High	Low	High	Low	High
Sarcoidosis	19	19.6	10.1	83.3***	40.0	100.0*	0.0	100.0
UIP	16	16.5	17.7	16.7	20.0	0.0	0.0	0.0
EAA	4	4.1	0.0	0.0	40.0*	0.0	66.7***	0.0
Others	58	59.7	72.2	0.0*	0.0*	0.0*	33.3	0.0
Total n	97		79	6	5	3	3	1

Data are presented as %, unless otherwise stated. UIP: usual interstitial pneumonia; EAA: extrinsic allergic alveolitis. *: $p<0.05$; ***: $p<0.001$ *versus* the respective *a priori* value. [#]: low (CD4/CD8 <3.5).

Table 10. – Probability of interstitial lung disease (ILD) as a function of lymphocytes and CD4/CD8 in suspected ILD when the percentage of granulocytes was low (eosinophils <2% and neutrophils <4%)

Lymphocytes % and CD4/CD8 ratio[#]	Subjects n	*A priori*	*A posteriori*					
			<30		30–50		>50	
			Low	High	Low	High	Low	High
Sarcoidosis	182	45.2	28.6***	86.1***	56.1	86.5***	33.3	55.6
UIP	25	6.2	9.4	5.6	3.5	0.0	3.0	0.0
EAA	35	8.7	1.4***	0.0	17.5*	2.7	39.4***	29.6***
NSIP	19	4.7	6.1	0.0	1.8	2.7	9.1	3.7
Connective tissue disease	10	2.5	2.4	0.0	1.8	0.0	6.1	7.4
Others	132	32.8	52.1***	8.3*	19.3*	8.1*	9.1*	3.7*
Total n	403		213	36	57	37	33	27

Data are presented as %, unless otherwise stated. UIP: usual interstitial pneumonia; EAA: extrinsic allergic alveolitis; NSIP: nonspecific interstitial pneumonia. *: $p<0.05$; ***: $p<0.001$ significantly different from the respective *a priori* value. [#]: low (CD4/CD8 <3.5).

Table 11. – Probability of interstitial lung disease (ILD) as a function of lymphocytes and CD4/CD8 in suspected ILD when the percentage of granulocytes was high (eosinophils $\geqslant$2% and/or neutrophils $\geqslant$4%)

Lymphocytes % and CD4/CD8 ratio[#]	Subjects n	*A priori*	*A posteriori*					
			<30		30–50		>50	
			Low	High	Low	High	Low	High
Sarcoidosis	57	18.6	13.9	44.4*	23.1	50.0*	21.4	0.0
UIP	87	28.3	34.2	22.2	11.5	6.3	0.0*	0.0
EAA	31	10.1	3.0*	5.6	34.6***	37.5***	50.0***	50.0
NSIP	27	8.8	7.8	16.7	15.4	0.0	14.3	0.0
Connective tissue disease	8	2.6	3.0	0.0	3.9	0.0	0.0	0.0
Others	97	31.6	38.1	11.1	11.5*	6.3*	14.3	50.0
Total n	307		231	18	26	16	14	2

Data are presented as %, unless otherwise stated. UIP: usual interstitial pneumonia; EAA: extrinsic allergic alveolitis; NSIP: nonspecific interstitial pneumonia. *: $p<0.05$; ***: $p<0.001$ significantly different from the respective *a priori* value. [#]: low (CD4/CD8 <3.5).

8.0 to 50.0% ($p<0.001$) when lymphocytes were >50% and granulocyte numbers were either low or high. Even the likelihood for NSIP increased from 2.9% to 9.1% with high lymphocyte and granulocyte numbers.

Discussion

This study presented an analysis of data from a cytological laboratory with an extended and diverse submission of BAL samples, in an attempt to quantify the information from differential cell counts, particularly in ILD. The results demonstrated that cell counts carried information that significantly altered the likelihood of a number of diseases. *A posteriori* probabilities, however, reached substantial values only in relatively frequent ILDs, in particular sarcoidosis, UIP and EAA. In the case of sarcoidosis, some cellular patterns even rendered this diagnosis the most likely among all.

The current data do not deny the value of BAL cell counts in terms of "fitting into the picture" in rare diseases, either by inclusion or by exclusion of diagnoses. The gain in likelihood of infrequent diseases could be great, but nearly all of them

remained unlikely after BAL analysis. It is obvious that the question, whether relative changes in probability or their absolute values *a posteriori* are more important, has no unique answer. Though not unexpected from a statistical point of view, the conclusion that cell differentials *per se* are most informative in frequent ILDs seems important to bear in mind when considering the interpretation of BAL results.

Many studies have demonstrated differences in BAL cell counts between lung diseases [2, 3, 9–11, 13, 14, 16, 17], but their variability, both biological and methodological, has always been found to be great. This results in a large overlap (table 2), which does not allow a reliable diagnosis in individual patients, irrespective of the statistically significant differences between groups. To the current authors' knowledge, it has never been described to what extent a specific BAL result changes the odds for a disease by comparing *a priori* and *a posteriori* probabilities within categories adopted from clinical usage. The current authors did not aim to derive predictions in individual patients, as it was believed that giving the likelihood for a disease, as well as its change, is better suited to the type of decision making used in the diagnostic process.

Individual predictions have been achieved by DRENT *et al.* [13] using discriminant analysis or (polychotomous) logistic regression [14]. In these two studies, patients with sarcoidosis, EAA and idiopathic pulmonary fibrosis were evaluated, as well as recovery, total cell count and biometric data, in addition to cell differentials. To find out whether the currently studied group of patients was comparable with the group studied previously, a linear discriminant analysis of the data set given in table 2 was performed, using only cell differentials. When random subsets comprising 50% of patients were used for computation and the other 50% for prediction, numbers were similar. Sarcoidosis was correctly recognised in ~ 90% of patients, EAA in 50%, UIP in 25%, BOOP in 10%, whereas NSIP was never identified. Taking into account the relative frequencies of diseases and the restricted set of variables, these findings indicate similar percentages of correctly classified patients and, thus, comparability of groups. The data also underline the conclusion that BAL cell counts *per se* convey significant information only in the most common ILDs. The exception was CEP (table 3), in which the significance of BAL has been demonstrated before [17].

To take into account prior information, separate analyses in three major groups of suspected lung diseases were performed. Not unexpectedly, the results regarding ILDs were most clear in the group that had ILD as a suspected diagnosis. It seems valuable to note that, with regards to frequent diseases, especially sarcoidosis, a similar pattern of likelihood emerged, even in patients with the suspected diagnosis of a lung tumour resembling the scenario of ILD, as well as those with suspected inflammatory disease.

It might be asked whether the results would have changed if more specific suspected diagnoses had been available, and especially whether rare diseases would have reached appreciable likelihood. Raising the likelihood for a rare disease upon entry also leads to higher absolute values *a posteriori*, even when the factor of change might decrease. Clinical data, however, are likely to be heterogeneous and incoherent, particularly in rare diseases, and it is unclear in which way these data can be used for maximising the information to be drawn from BAL. The amount of data needed to derive safe conclusions seems enormous. Although, in the majority of patients, the prior information available to the current authors was limited to the categories of suspected diagnoses, as were used, it was considered unlikely that more specific information would have changed the picture. Clinical experience suggests that differences between suspected and final diagnoses occur at least as often in rare as in frequent diseases. In addition, it has to be considered that the choice of a rare disease as a suspected diagnosis is highly dependent on the clinical environment and experience.

The cytological criteria used for the evaluation of data were similar to those of previous BAL studies [12, 16]. The current results, from univariate to trivariate analyses (tables 3–11), were fully consistent with the disease-specific patterns reported previously [2, 3, 9–14, 16–18]. The criteria were not varied because an attempt to find statistically reliable estimates of optimal cut-off values would need much extended data sets. This is possibly an issue of future multi-centre studies.

The four major diseases, sarcoidosis, UIP, EAA and NSIP, covered ~60% of all ILDs. The remaining 40% were represented by a wide variety of less frequent or rare ILDs, as well as inflammatory diseases and tumours. To check whether the current results were biased by the specific distribution of diagnoses among the samples, additional analyses were performed, in which the data set was enriched or depleted with regard to the relative frequency of a disease. This was done only for sarcoidosis, as the major ILD, and achieved by randomly omitting patients with diseases other than sarcoidosis or sarcoidosis, respectively. At frequencies between ~20 and 40%, the pattern of relative changes in likelihood, as shown in tables 3–11, was not qualitatively altered, although, of course, absolute numbers changed. Therefore, it is believed that the result did not critically depend on specific characteristics of the current authors' laboratory, such as the distribution of final diagnoses. Reliable information on smoking was available only in a minority of patients. Smoking is a well-known factor of influence in some rare ILDs, such as RBILD and histiocytosis X, but remarkably the changes in likelihood were parallel in the total group and in smokers, at least as far as major diagnoses, such as sarcoidosis, EAA and UIP, were concerned (tables 6 and 7). This suggests that the current authors' arguments regarding the informative content of BAL counts are not dependent on smoking history, thus specific threshold values might do so.

BAL differential cell counts are not specific markers of diseases. Only under certain conditions can a specific diagnosis be obtained solely from BALF, *e.g.* by detection of siderophages as a marker of alveolar haemorrhage, or of infectious organisms or tumour cells. Such analyses take into account specific cytological characteristics, in addition to cell differentials. At the same time, however, it must be stated that these instances represent only a minor fraction of indications under which BAL is performed. On account of this, and for achieving a sufficient number of cases, the analysis was restricted to cell differentials, and the conclusions are limited to these.

In addition to standard cell differentials, the CD4/CD8 ratio was evaluated, which has been introduced as a valuable marker in the diagnosis of ILDs, particularly in sarcoidosis [9–12]. The current authors' data are fully consistent with this. The likelihood of sarcoidosis was raised by more than a factor of two by a high CD4/CD8 ratio (table 5). If used alone, this marker was superior to lymphocyte and granulocyte numbers in the diagnosis of sarcoidosis (tables 3–5). Similar changes could only be reached if these two variables were used in combination (table 6). When the CD4/CD8 ratio was combined with lymphocyte and granulocyte numbers, the *a posteriori* probability of sarcoidosis could be tripled and exceeded 85% (table 10). Notably, when three categories were chosen for CD4/CD8, one of them comprising a ratio <0.5, there was no apparent benefit in the prediction of EAA *versus* sarcoidosis.

In summary, the data of the present study suggest that bronchoalveolar lavage differential cell counts *per se* contain

substantial diagnostic information only in frequent interstitial lung diseases, with considerable and meaningful changes in *a posteriori* probabilities. In more rare diseases, the potential diagnostic value of bronchoalveolar lavage cell differentials appears to be highly dependent on additional clinical information.

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