

Interpretation of bronchoalveolar lavage fluid cytology

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Preface

Bronchoalveolar lavage (BAL) explores large areas of the alveolar compartment providing cells as well as non-cellular constituents from the lower respiratory tract. It opens a window to the lung. Alterations in BAL fluid and cells reflect pathological changes in the lung parenchyma. The BAL procedure was developed as a research tool. Meanwhile its usefulness, also for clinical applications, has been appreciated worldwide in diagnostic work-up of infectious and non-infectious interstitial lung diseases. Moreover, BAL has several advantages over biopsy procedures. It is a safe, easily performed, minimally invasive, and well tolerated procedure. In this respect, when the clinician decides that a BAL might be helpful to provide diagnostic material, it is mandatory to consider the provided information obtained from BAL fluid analysis carefully and to have reliable diagnostic criteria. Therefore, the interpretation of BAL fluid cytology has to be standardized to improve the diagnostic power.

It is with this background that the concept for this CD-rom was developed. It is aimed mainly at clinicians who are having to deal with the diagnostic problems of patients with diffuse interstitial lung diseases. The introductory sections summarize the history of BAL. Furthermore, the importance of standardization of handling BAL samples will be discussed. Additionally, BAL fluid cytology features are presented and the interpretation of the BAL fluid cell differentials is discussed.

Recently, a validated computer program based on logistic regression analysis using BAL fluid analysis results to distinguish between the three most common interstitial lung diseases: sarcoidosis, idiopathic pulmonary fibrosis, extrinsic allergic alveolitis or drug-induced pneumonitis was developed. One of the limitations of this program was that it was not useful in discriminating between infectious disorders and non-infectious disorders.

An updated windows 2000 version of this validated computer program - thought to improve the diagnostic power of BAL fluid analysis - is presented.

It is hoped that this CD-rom will be a source of reference in the work-up and interpretation of BAL fluid cytology to everybody involved in the management of patients suffering from diffuse interstitial lung diseases or with suspected pneumonia.

Although details hidden in BAL fluid may add useful information about a patient's disorder, the results should be considered in the context of other information from conventional investigative methods and the individual's unique history. To establish the diagnosis a thorough history is essential as it may identify a potential aetiological factor.

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Foreword

Bronchoalveolar lavage (BAL) has become a widely applied diagnostic tool in pulmonary medicine. This holds true for both infectious and non-infectious infiltrative and immunological lung diseases. Barriers which tried to restrict the use of BAL to research application and to put down its clinical value have finally been overcome: In two recently published international statements (ATS, ERS, also WASOG) on the major interstitial lung diseases, BAL was considered to be helpful in strengthening the diagnosis in a sarcoidosis patient without biopsy; BAL and/or transbronchial biopsy were considered as a requirement to exclude other diseases in a patient with idiopathic pulmonary fibrosis/UIP who does not undergo surgical biopsy (one of the four major criteria for making a clinical diagnosis of the disease).

BAL studies should not be limited to counting the cell differentials only. At least as important as looking at cell differentials is to observe the morphological appearances of cells and particles. Examples are the different morphology in extrinsic allergic alveolitis (foamy macrophages, heterogeneous macrophage size, presence of plasma cells) versus that of sarcoidosis (more monomorphous appearance of macrophages, less activated lymphocytes), the presence of malignant cells, the characteristic features of alveolar proteinosis, or dust particles such as asbestos bodies, and other features.

Also, it is important to consider BAL cell differentials not in isolation but in the context of the clinical setting and the radiological, particularly the HR-CT appearance of the disease. For example, if the CT scan shows a patchy ground glass pattern, BAL may be able to reveal that this patient suffers from extrinsic allergic alveolitis (high lymphocyte count), or a smoking related respiratory bronchiolitis/interstitial lung disease (high smoker's macrophage count and normal cell differential), or alveolar haemorrhage (high count of haemosiderin laden macrophages).

This CD-rom interpretation of BAL fluid cytology is exactly using such an approach. The work provides a detailed insight into the specific morphologic features of BAL and the interpretation of the BAL cell differentials and also presents illustrative cases with their clinical profile and characteristic imaging findings.

We hope that this work will find a warm appreciation amongst chest physicians, pathologists, laboratory technicians, in short, amongst everybody involved in the work-up and interpretation of BAL fluid cytology.

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Introduction: Importance of standardization of handling BAL fluid samples

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The concept of bronchoalveolar lavage (BAL) is not new. Bronchial lavage was a technique performed during rigid bronchoscopy, especially for obtaining samples in patients with tuberculosis. To sample the alveolar space, FINLEY described the use of the Matras catheter (used to perform bronchograms without a bronchoscope) to instill and immediately aspirate fluid [1]. However, the use of a flexible bronchoscope to obtain BAL fluid (BALF) samples was popularized by Dr. HERBERT REYNOLDS [2]. The technique has been widely used for both research and diagnostic indications.

Technical aspects

The technical aspects of the lavage procedure can not be ignored. Some of the controversy about interpreting lavage results can be traced to different centers having different results because of variations in the technique. As in any test, the details are important. This work deals with the cellular aspects of BAL. In order to interpret the cellular information, one should be sure that the information has been obtained in a reliable manner. I will review some of the difficulties which have been described with various aspects of lavage.

One of the difficulties inherent with BAL is the variability of the sampling [3]. The lavage process provides a washing of the alveolar space. In the process of washing the space, the amount of cells and fluid returned will vary. This variability may be on the basis of the underlying disease. Other factors also affect the variability: the amount of fluid instilled [4,5], the amount of pressure used to aspirate the fluid, the method of collecting the aspirated fluid [6], and the handling of the aspirated fluid. In addition, different parts of the lung may have different BALF findings [7,8]. For *Pneumocystis carinii*, lavage in the upper lobe may contain far more organism than lavage from the middle or lower lobe [9]. Some of the differences are due to operator differences, some are due to the conditions when the procedure is done.

To deal with the variability of lavage, several groups have proposed using markers [10]. These include external markers, such as methylene blue [11], or internal markers, such as urea [12]. Unfortunately, none of these markers is perfect. This is because the alveolar space is not a water tight vessel. During the process of lavage, fluid and proteins are crossing from the lung fluid into the cells and blood stream. Also, internal proteins efflux into the lung fluid [3]. Because of this variability of lavage, a recent recommendations of an European Respiratory Society Task Force was to report acellular content of BALF per ml of aspirated fluid [13].

Standardization of other aspects of BAL have been attempted. In the United States, KING *et al.* demonstrated that BAL interpretation could be improved by using a standard approach across several centers [14]. However, those recommendations have not been widely followed. The European Respiratory Society (ERS) had made a series of recommendations on performing BAL [15,16]. In a recent report, the ERS has also included recommendations regarding the reporting of BALF analysis results. These recommendations are summarized in Table 1 [13].

Table 1. - Causes of variability and recommendations for obtaining and processing BALF*.

Source of variability	Recommendation
Disease process itself	Specify underlying disease
Dwell time to aspirate fluid	Keep to a minimum and specify dwell time, especially if prolonged
Suction pressure during aspiration	Keep to a minimum (25-100 mm Hg)
Physician doing lavage procedure	Specify
The handling of lavage fluid (e.g. filtered versus not filtered, concentrated)	State which technique
Volume of instilled fluid	Use at least ≥ 100 mL instilled volume and report volume instilled (ERS Task Force recommended 200-240 mL [16])
Number of aliquots	Specify and standardize (ERS Task Force recommended four [16])
Position of patient	Specify
Area which is lavaged (e.g. one versus two lobes, middle lobe versus lower lobe, other)	Specify
Variability of return of lavage fluid	Report volume and percentage of fluid recovered Established minimum percent recovery
Reporting measurements of acellular components	Standardly report values per mL of BALF recovered (as well as any other special approaches)
Handling of BALF, including if handle the first aliquot separately from rest	Specify

* Modified from European Respiratory Society Task Force report [13].

Bronchoalveolar lavage fluid cytology

The cytology of BALF is an analysis of the cellular components of the specimen. The most abundant cells retrieved by BAL are the inflammatory cells which line the alveolar space. These include the macrophage, lymphocyte, and neutrophil. The alveolar macrophage is the most common cell in the BALF. In a normal subject, it represents more than 80% of the cells retrieved. The lymphocyte represents approximately ten percent of the cells retrieved. In the healthy smoker, up to five percent neutrophils may be seen. In the healthy nonsmoker, neutrophils are rarely seen in the BALF [14,15].

The normal values for the percentage of lymphocytes and macrophages varies between laboratories. This is in part because the preparation of the of the slide can lead to a variation of proportion of lymphocytes. This includes the use of a cytocentrifuge versus membrane to prepare the slide [17]. For the cytocentrifuge, the speed of the centrifuge, the area of the slide, and the number of cells counted have all been shown to lead to differences in counts [18]. In addition, the cells can be sometimes difficult to distinguish using the standard Giemsa based stain. In active inflammatory diseases, such as sarcoidosis, monocytes recruited into the lung can look like activated lymphocytes. Nonspecific esterase is another way to identify macrophages, and flow cytometry with a pan-lymphocyte marker can count lymphocytes [19]. However, these are time consuming stains and usually not necessary. The variation in cellular differential seems to be greater between laboratories than within the same laboratory [14]. However, there are no standards which have been used to certify laboratories.

Diagnostic value of bronchoalveolar lavage

Cytologic examination has also been used to detect malignancy. This technique is fairly standard and criteria for malignancy used for other bronchial samples can be readily applied to BALF samples [20]. For some conditions, such as lymphangitic spread of breast cancer, BAL has been quite useful in providing a diagnostic sample [21,22]. For patients with bronchoalveolar cell carcinoma, BAL may have the highest diagnostic yield [20].

BAL has become an accepted technique for diagnosis of infection [23,24]. This includes opportunistic infections, such as *P. carinii* [25]. The diagnosis of *P. carinii* usually requires direct visualization of the organism, since it does

not grow in culture. Several stains have proved useful in detecting *P. carinii*. The standard stain for detecting *P. carinii* has been a silver stain, such as the Methenamine stain [26]. There are more sensitive stains such as the immunofluorescent stains [27,28] and even polymerase chain reaction has been employed [29]. These more sensitive, and expensive techniques have not proved necessary for the evaluation of most immunosuppressed patients when examining BALF. They are usually reserved for specimens with a lower number of organisms, such as induced sputum and bronchial wash [27-29]. The Wright-Giemsa and similar stains are used for defining cellular morphology. A modified Wright-Giemsa can be done within five minutes. It has been shown to detect *P. carinii* in the BALF of over 70% of HIV infected patients with *P. carinii* pneumonia [30]. It is not as sensitive in non-HIV infected patients, who have a smaller number of organisms [30]. The technique of BAL does have an effect on the yield for *P. carinii*. The volume of instilled fluid does not appear to be crucial, since the number of *P. carinii* organisms is relatively constant during sequential BAL, with increasing volumes of BALF instilled [31]. On the other hand, the area in which BAL is performed has proved important [32]. Multiple lavages or lavage directed to the upper lobe or area of most diseases has enhanced the yield of BALF for diagnosing *P. carinii* pneumonia [8,9]. Beyond the organism itself, the cellular differential count has clinical information. Increased neutrophils or eosinophils are associated with a worse prognosis [33].

Bacterial infections may also be diagnosed by BAL. These include unusual bacterial infections such as Legionella [34]. BAL has also been used to diagnose routine bacterial infections. For the diagnosis of routine infections, one needs to perform semi-quantitative cultures of the BALF [35]. The rationale behind semi-quantitative cultures was to separate colonization from deep seated infection. Semi-quantitative cultures have proved useful for evaluating BALF samples for both ventilated [36] and non-ventilated patients [37]. In addition to the standardized BAL procedure, it is important to culture the fluid in a standard manner. Recommendations have been made for the technique of BALF cultures [38]. The examination of the cells in bacterial pneumonia usually demonstrates a marked increase in neutrophils. This is not specific. The use of Gram stain to identify bacteria was shown to be useful in diagnosing pneumonia [35]. Subsequently, CHASTRE proposed counting the number of cells with intracellular organisms (ICO) [39]. The diagnostic importance of cells with ICO is still unclear [36].

In conclusion, the analysis of cells obtained by BAL can be quite valuable clinically. To improve the information, one should educate themselves on what they are seeing. This CD-ROM provides excellent examples of the types of cells one can see and how to integrate the BAL findings with the clinical presentation.

The cellular analysis of the BALF relies on an accurate counting of the various cells involved. Care should be taken to identify the cells, and differential counting should be done consistently. Although universal standards are not yet available, it seems obvious that each institution should be sure that the various readers of BALF samples have agreement about their BALF differential counts [19]. Using a standard technique for performing the BAL and handling the sample will also reduce variability.

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History: Visualization of the cells in BALF has directed clinical lung research

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Sampling the lower respiratory tract by bronchoalveolar lavage (BAL) to retrieve detachable airway and alveolar cells and to collect non-cellular soluble proteins and other substances has been invaluable for stimulating research of the normal lung and of the diseased lung in patient-volunteer participants [1]. This has been performed much more easily and safely since the availability of fiberoptic bronchoscopy [2]. Although BAL analysis has contributed to the scientific understanding of the healthy and disease-afflicted lung, it has had helpful clinical applications as well [3-11]. Material on the present CD-ROM, an update of prior work published by Dr. DRENT and colleagues [12] about the use of BAL analysis in the diagnosis of interstitial lung diseases (ILD) has been extended to further differentiate BAL cellular findings in infectious illnesses from other ILD. It is a pleasure to offer a historical perspective about obtaining lung cells, when the technique of fiberoptic bronchoscopy was just becoming widely utilized about 30 years ago [13-15], and how this stimulated me and my clinical colleagues to investigate the normal respiratory tract and the immunopathology associated with several forms of ILD.

A good picture may be worth a thousand descriptive words about the appearance of lung cells, and more importantly what they may have been doing *in situ* - behaving and doing their usually thorough and competent job of respiratory host defense [16,17], or causing mischief and creating or perpetuating illness [18]? Looks, however, may be deceiving, as activated-appearing cells may be responding appropriately by secreting cytokines, processing antigens, and stimulating immune reactions, or attracting inflammatory back-up cells [19]. A picture must be extrapolated to a reasonable clinical setting and a feasible patient diagnosis.

The ease and safety of fiberoptic bronchoscopy, coupled with BAL for retrieval of airway-alveolar cells that can be detached and washed out of the distal airways or lavaged selectively from the bronchial surface (or brushed off), and also combined with tissue biopsies (transbronchial and endobronchial) - all together allow for a comprehensive, direct, interior view and sampling of the respiratory tract. This has provided tremendous insight into the cause and immunopathogenesis of many airway and parenchymal respiratory diseases and has facilitated patient diagnosis. The capability of using normal volunteer subjects for bronchoscopy and BAL has been of great help in establishing normal values. Thus, pulmonologists can have direct visualization out into the small conducting airways [20], perform BAL and obtain tissue from the lower respiratory tract, a combination that is unique and not readily available from most other organ systems, except perhaps the urogenital and gastrointestinal tracts and large skeletal joints (knee). This direct interior 'view' is also different from those achieved with radiographic and scan imaging.

In my clinical research experience, four distinct and memorable instances of an initial view of BAL retrieved lung cells were especially impressionable. Each stimulated further investigation about why certain cells were grouped or associated together: 1) normal human alveolar macrophages (AM) and the few lymphocytes that accompanied them in BAL fluid (BALF); 2) rosetted lymphocytes stuck to the surface of alveolar macrophages from a patient with pulmonary sarcoidosis, 3) the foamy appearance of alveolar macrophages that were almost engulfed by the numerous surrounding lymphocytes obtained from a patient with hypersensitivity pneumonitis, and 4) the presence of polymorphonuclear neutrophils (PMNs) and eosinophils mixed among AM in BALF from patients with idiopathic pulmonary fibrosis (IPF). These 'sightings' occurred over a brief period, after we began to do BAL on normal human volunteers [21] and patient participants with forms of interstitial lung diseases [22,23]. For me, the observation of lung cells has always been a two-fold process - initially looking at a wet cell preparation [21] taken from the freshly centrifuged BAL fluid, revealing viable cells and the glitter of intracellular inclusions or granules, and then counting the traditionally stained cell preparation. Personally, I have always felt more confident of my wet prep differential cell count for cell enumeration than the cytocentrifuge or filter preparation of stained cells. Certainly nothing can give the excitement or thrill of seeing the living cells and feeling their energy and interaction. Living lung cellular biology is the only description that comes to mind and pen.

Normal alveolar macrophages and respiratory lymphocytes

Following lavage recovery of rabbit macrophages [24] for *in vitro* study, research interest peaked to obtain human lung cells initially from normal volunteer participants through a variety of endobronchially placed catheters (reviewed Ref. 1) and then with fiberoptic bronchoscopy [25,26]. Among the population of respiratory cells from normal young non-smokers, most of the cells were alveolar macrophages but about 15% were lymphocytes plus a few PMNs or other inflammatory cells [21]. Considerable interest was focused on the AM as a phagocytic cell, and we too were eager to study its immune receptors [27] and antibody-mediated uptake of opsonized bacteria [28]. Alveolar macrophages seemed to exist in various sizes as recovered in normal BAL, suggesting subpopulations of them with perhaps different functions or representing various stages of development [29,30]. The intent was to find ways to manipulate lung humoral immune responses that might improve the efficiency of host defenses to cope with microbes entering the lower respiratory tract.

Overlooked were a very small population of other macrophages now identified as dendritic cells (also Langerhans' cells) [31], the more efficient antigen presenting cell so effective in initiating cellular immune responses in the lung [32-34] or increased by smoking [35,36] and associated with certain diseases [37-39].

Others [4,40-42] were attracted to the lymphoid cells initially, and information obtained about cellular immunity stimulated by lymphocytes became important in many diseases such as sarcoidosis, hypersensitivity pneumonitis, occupational diseases and respiratory infections such as HIV.

Sarcoidosis: adherence of lymphocytes to alveolar macrophages

In a fresh wet mount BAL cell preparation, viewed under phase contrast microscopy, from a patient with active sarcoidosis [3], the appearance of lymphocytes stuck to the surface of AMs is striking; the cells adhere and do not fall off and are not phagocytosed by the AM. A cytospin stained cell preparation makes these rosettes easy to notice. Occasionally, a spontaneous rosette can be found among BAL cells from a normal smoker or non-smoker, and YEAGER and colleagues [43] found that 1.8-2.1% of AM had one or more adherent lymphocytes. In their group of 14 sarcoidosis patients, macrophage rosettes involved 8-11% of the AMs. We found 4 of 17 BAL specimens from sarcoidosis patients had lymphocyte-macrophage rosettes [44]. The implication of these rosettes is still speculative, but HUNNINGHAKE and colleagues [5, 45,46] found that the lymphocytosis in BALF from many patients with sarcoidosis was comprised mainly T-cells which were activated. T-cells were important in creating the alveolitis that probably preceded granuloma formation. Lung T-cells release a pertinent cytokine in the cellular activation mechanism(s) of sarcoidosis [47]. Certainly, T-cell immune mechanisms have become the research focus of many investigators.

Lung cells in hypersensitivity pneumonitis

This has seemed to be the perfect respiratory disease to study with distinct episodic clinical features, often a defined antigen that stimulates specific humoral responses (precipitating antibodies), striking parenchymal lung involvement that is at first acute but can become chronic and persist (often establishing host tolerance when repeated antigen exposure continues), and decidedly immunologic in nature (but not allergic) [48-50]. Performing BAL analysis on the first patients we encountered [22] was fascinating - all the ingredients were present in BALF between the alveolar cells and the lavage supernatant fluid (and contrasted with the patient's serum) to fit the immunologic response together.

The patient's clinical history usually gave good clues to the kind of antigen(s) encountered in his/her environment or workplace. A specific precipitating antigen-antibody reaction could be found in serum and occasionally in BAL fluid (for 3 of the initial 7 patients studied [23]), which was in the IgG fraction in BALF. Although IgM levels were detected in BALF, no precipitating antibody activity was found. IgE and two complement components, C₄ and C₆ were in normal amounts in BALF. The other surprise was the composition of the BAL cells. Lymphocytes predominated in the cellular count, a mean count of about 62%, and the majority were T-lymphocytes (mean 70%), and about 6% were B-cells (assayed with sheep erythrocyte forming rosettes). Alveolar macrophages, reciprocally reduced in proportion to the high percentage of lymphocytes, were about 29% of the total lavage recovered cells. Within the macrophage population, larger than usual size macrophages were seen with vacuolated and opaque appearing cytoplasm and few granules; these were considered 'foamy' or engorged macrophages [51].

Importantly, when subjects with hypersensitivity pneumonitis (9 of 10 had pigeon or bird fancier's lung disease) were challenged with inhaled diluted pigeon serum by FOURNIER and colleagues [52] and re-evaluated by BAL 24 hours after inhalation and again five or eight days later, the cellular findings in BALF were quite different from the baseline lavage results, obtained five days prior to challenge. For the ten HP patients, the baseline lymphocyte percentage was on average 60% and PMN 8.3% (range 0-31%); 24 hours after challenge the total cells recovered in BALF doubled with the lymphocyte percentage constituting about 38%, but PMNs now accounted for an average of 41%. Within five to eight days, counts returned to baseline for the six subjects who underwent a third lavage study. This acute inflammatory cell response of PMNs after antigen challenge seemed like an Arthus response or Type 3 reaction [48,49].

It was important that LEATHERMAN and colleagues [53] more precisely determined the BAL T-cell phenotypes in hypersensitivity pneumonitis as largely CD8 cells (T-suppressor/cytotoxic cells) in contrast to the high percentage of CD4 T-cells found in sarcoidosis. Subsequently, others [54] found that patients with an insidious onset of hypersensitivity pneumonitis and development of pulmonary fibrosis could have a relative increase in CD4 T-lymphocytes in BALF *versus* a non-fibrosis group with increased CD8 T-cells, raising the possibility that CD8T-cells might have a protective effect on developing fibrosis. This is relevant to the long term effects of continued antigen exposure (continued farming or pigeon contact) that has been thoroughly studied by CORMIER and colleagues in Quebec, Canada.

Asymptomatic dairy farmers with serum precipitins to *Micropolyspora faeni* underwent BAL [55] for cellular and immunoglobulin analysis. Six of ten farmers had an increased number of cells in BALF and a high percentage of lymphocytes (mean 52.5%); also, four of the six had positive precipitins in BALF. Thus, normal farmers may have BAL findings consistent with an immunologic alveolitis. When these asymptomatic farmers were re-evaluated two years later [56], and then six or seven years later [57], 33 of 43 original farmers were re-evaluated but this did not include another BAL. Only five of the 33 were no longer farmers by profession. However, original lavage findings of an elevated percentage of BAL lymphocytes did not predict later who would develop functional lung deterioration or extrinsic allergic alveolitis. Therefore, the respiratory system of many persons can modulate the impact of repeated and continued foreign aerosol antigen exposure by developing a subclinical cellular immune response that may not progress to overt illness, thus their lungs can coexist or become tolerant to an environmental antigen/substance. In other subjects this exposure can create symptomatic lung disease. Allergy in the typical fashion does not develop either. Much can still be investigated about this occupational illness of defined cause (often) that provides a window into the subtle mechanisms of respiratory immune responsiveness.

Idiopathic pulmonary fibrosis: a mixed inflammatory cell response in BALF

Considerable clinical and research interest has existed for forms of diffuse interstitial pneumonia causing fibrosis, acutely or more chronic in development. This was probably rekindled with a presentation by Drs. LOUIS HAMMAN and ARNOLD R. RICH [58] of four cases of fulminating fibrosis seen and studied at the Johns Hopkins Hospital between 1931-35. Later, several prominent pulmonary clinicians and pathologists [59,60,61] reported on series of patients that elucidated the clinical course, histopathology and treatment of ILD. In this context, a task force, directed by Dr. CLAUDE LENFANT, to develop a lung research program for the National Heart and Lung Institute of the National Institutes of Health, published in 1972 [62], recommended the study of diffuse fibrosis of unknown causes and acquired connective tissue diseases to identify etiologic agents, and assess other risk factors such as genetic constitution and altered immunologic mechanisms. With this mandate the Pulmonary Branch's research agenda, directed by the Branch Chief, Dr. RONALD G. CRYSTAL, began a concerted effort to investigate interstitial lung diseases. A patient protocol was developed by Dr. CRYSTAL and Dr. JACK D. FULMER, co-principal investigators, in late 1974 and patients were enrolled soon after. This project on diffuse fibrotic lung disease was a correlative study of the etiology, pathogenesis, pathophysiology and therapy of these patients. Patient participants would receive a fiberoptic bronchoscopy with BAL performed by the Pulmonary Branch Staff physicians; BALF cellular and protein analysis were done by Dr. REYNOLDS, a collaborating investigator located nearby in the Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases at NIH. The protocol's enrollment of patients moved quickly, and the interesting BALF analyses were reported less than a year later at an NIH Combined Clinical Staff Conference, November 13, 1975 [22] and published later in more detail [23]. The first decade's work on ILD was best reviewed by Dr. CRYSTAL and colleagues [63] as a medical progress report in the

New England Journal of Medicine.

In contrast to normals including smokers [21], the cellular differentials from the IPF patient participants [23] were quite different - notably PMNs (mean percentage 33 in stained differential count) and eosinophils (mean 2.7%) were increased from seven IPF patients not receiving corticosteroid therapy. For 12 patients on therapy, the PMN percentage was less (mean 14%), but still higher than normal controls (< 3%), and the eosinophils were still increased (mean 3.2%). Cellular counts of lymphocytes (mean 12.1%) in untreated and treated patients (mean 7.7%) were within the range of normals. The macrophages which comprised the remaining cells were not remarkable in appearance.

The surprise about IPF BAL cells was an apparent mixed alveolar inflammatory reaction of PMNs and of some eosinophils. Both of these cell types subsequently received research attention. HASLAM with colleagues [64] analyzed BAL cells from patients (n=36) with lone cryptogenic fibrosing alveolitis [60], which is quite similar to IPF, and other patients (n=15) with associated connective tissue diseases. They found for lone CFA subjects that the mean BALF PMN percentage was 13% and the eosinophil percentage was about 6% (over 2.4% for 19/36 patients). Other studies (65,66) continued to analyze these cells and correlate them with response to treatment and prognosis (reviewed in [1]). The neutrophilic alveolitis characteristic of many patients with IPF also stimulated research for chemotactic stimuli that might attract PMNs into the lungs [67]. Subsequent identification of a proinflammatory cytokine, interleukin-8, derived from alveolar macrophages is involved in the process [68,69].

In conclusion, identification of cells in BAL obtained from normal people and those with a variety of lung diseases has helped define the milieu of the lower respiratory tract which has promoted research of the specific function(s) and interactions that occur to maintain health and what changes in the composition and activity of cells that occurs with illness. Although much has been described, more is still left to uncover and learn to prevent development of illness.

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Bronchoalveolar lavage

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Summary

Bronchoalveolar lavage readily explores large areas of the alveolar compartment. After the introduction as a research tool, bronchoalveolar lavage has been appreciated extensively for clinical applications in the field of opportunistic infections and interstitial lung diseases. It is considered as a safe, minimally invasive procedure, associated with virtually no morbidity. In selected cases, bronchoalveolar lavage is useful for establishing or ruling out a diagnosis with only a low risk of incorrect diagnosis. In contrast, the role of bronchoalveolar lavage in the management and prediction of the prognosis of a certain disorder is, so far, rather controversial. The potential practical value of BAL in the diagnosis of diffuse interstitial lung diseases will be discussed.

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Introduction

Diffuse interstitial lung disease (DILD) poses a significant challenge for the clinician because the aetiology is often unknown. To establish the diagnosis, a thorough history is essential as it may identify a potential aetiological factor (e.g. drug reaction, subtle or prolonged environmental and/or occupational exposures). Pulmonary diseases have traditionally been evaluated by laboratory tests, lung function tests, imaging procedures and tissue biopsies [1-3]. Bronchoalveolar lavage (BAL) represents an additional tool in assessing the health status of the lung. BAL is a procedure in which the bronchoalveolar region of the respiratory tract is lavaged or washed with an isotonic salt solution. It is a method for sampling cells and solutes from a large area deep within the tissue of the lung. BAL has emerged to be useful both in fundamental research and for clinical purposes [4-9]. Clinically, this procedure was first used at Yale in 1922 as a therapeutic tool, e.g. in the management of phosgene poisoning and as a means of removing abundant secretions [4]. Gradually, BAL became more widely applied in the treatment of various pulmonary disorders, such as cystic fibrosis, alveolar microlithiasis, alveolar proteinosis and lipoid pneumonia [4-10]. The application of BAL for diagnostic purposes has significantly improved the diagnostic work-up of DILD [4-14]. It is broadly indicated in patients with diffuse chest radiograph abnormalities, whatever the suspected cause. The underlying disorders may be of infectious, noninfectious immunological, malignant, environmental or occupational aetiology. Even in conditions in which lavage is not diagnostic, the results may be inconsistent with the suspected diagnosis, and then focus attention on more appropriate, further investigations. For example, even a normal lavage may be useful to exclude some disorders with high probability.

Furthermore, BAL has the potential to be useful in the assessment of disease activity, in determining prognosis [15], and in guiding therapy. These are, however, the most critically discussed topics in the field of BAL [9, 11, 15-18].

Bronchoalveolar lavage procedure

The BAL technique is not completely standardized. Details of the different steps of the procedure used may vary a great deal between laboratories. Sometimes these differences give rise to problems when comparing data. Attempts have been made to set up a frame-work for the different steps of the procedure, such as the amount and temperature of fluid injected, the number of aliquots used, the "dwelling time" and the aspiration pressure. Guidelines and recommendations for using a standardized approach regarding the procedure as well as processing the material have been published [5, 7, 8, 13, 19].

In general, after local anaesthesia of the larynx and bronchial tree with lidocaine to control cough, BAL can be performed through a bronchoscope transnasally, transorally, or through an in-place tube. After a complete inspection of the airways, a fiberoptic bronchoscope is gently impacted, or "wedged", into a segmental or subsegmental bronchus [7, 13]. From an anatomical point of view, either the middle lobe or lingula is most convenient to access, and, therefore routinely used [13, 19]. From these lobes, 20% or more fluid and cells are recovered compared to the lower lobes. The basic goal is to ensure that the injected fluid reaches the appropriate pathological area. In addition, the aspirate has to be a representative sample containing solutes and cells obtained from the lower respiratory tract, reflecting the pathophysiological process of the disease. In general, results obtained at one site are representative for the whole lung especially, in DILD, lavages of separate segments yielded similar results. However, in localized disease such as inflammatory infiltrates and malignant lesions, lavage at different sites may provide different results [13]. In these localized disorders, it is recommended that the area of greatest abnormality, demonstrated on the chest radiograph or high resolution computed tomography (HRCT) scan, be chosen as an appropriate segment for BAL [13].

The most commonly used lavage solution is sterile isotonic saline, prewarmed to body temperature. The fluid is instilled into the subsegment through the biopsy channel of the bronchoscope and, subsequently, immediately aspirated and recovered. Aspiration should be performed by applying gentle suction. Suction which is too forceful can cause collapse of the distal airways or trauma of the airway mucosa, and so alter the BAL fluid (BALF) profile

[7, 13]. The major technical variation in the BAL procedure is the fluid volume used. Usually, the total volume infused ranges from 100-300 mL using multiple aliquots of 20-50 mL. Reports by various groups performing lavage with different amounts of saline suggest that, at least in healthy individuals, the information about cell types obtained in volumes ranging from 100-250 mL are comparable [8].

Side-effects of bronchoalveolar lavage

BAL is a safe, noninvasive and generally well-tolerated procedure [7, 9, 13]. Only one report was found describing a patient who died with a picture consistent with acute pulmonary oedema and septic shock following BAL [20]. The authors hypothesized that the fulminate deterioration in this patient was caused by a massive release of inflammatory mediators, resulting in hypotension, pulmonary oedema and, finally, multiple organ failure.

Side-effect	Occurrence
Fever	10%-30%, some hours after BAL
Inflammatory response	Transient increase in the lower respiratory tract neutrophils, resolving within 72 h
Bronchospasm	Rarely in normoreactive, more frequent in hyperreactive patients
Wheezing	In hyperreactive patients up to 1-2 weeks
Crackles	Within 24 h over dependent areas
Lung function decrement	Transient decrease of FEV ₁ , VC, PEF, Pa,O ₂ Transient rise of Pa,CO ₂ in patients with COPD
Chest radiograph abnormalities	Radiographic infiltrates 30 min following BAL (90%) of which the vast majority are resolved within 24 h
Epithelial integrity change	No effect on lung epithelial permeability 24 hours after BAL Transient decrease of ciliary beat frequency
Bleeding	Not significant
Heart rhythm irregularity	<2%

FEV₁: forced expiratory volume in one second; VC: vital capacity; PEF: peak expiratory flow; Pa,O₂: arterial oxygen tension; Pa,CO₂: arterial carbon dioxide tension; COPD: chronic obstructive pulmonary disease.
Source: Modified from Klech and Pohl [13]; and Baughman [7]

Most of the reported side-effects are closely related to the endoscopic procedure, and, the volume and temperature of the instilled fluid [7, 13]. Common complications associated with the lavage itself include coughing during the procedure, transient fever, chills and marked malaise occurring some hours after the performance of the BAL (table 1). The overall complication rate of BAL was reported to be < 3%, compared to 7% with transbronchial biopsy and 13% when performing open lung biopsy [7, 9].

Processing of BALF

The obtained cells can be evaluated by cytological techniques as well as by immunohistochemical procedures. Routine processing includes the analysis of total and differential cell counts, and when possible, the determination of lymphocyte subsets by monoclonal antibody techniques. In addition to the usual May-Grünwald Giemsa staining, special stains and culture of BALF samples have increased the accuracy of diagnosing opportunistic infections in immunocompromised patients [21]. Furthermore, BAL can also be used as a tool for confirming pulmonary infections in the nonimmunosuppressed host, in particular in mechanically ventilated patients, e.g. ventilator associated pneumonia (VAP) [22-24]. Additionally, enumeration of May-Grünwald Giemsa stained cells containing intracellular micro-organisms (infected cells) appeared to be useful in assessing the diagnosis VAP [23, 24]. Depending on the population studied, the reported cut-off values ranged from 2-25%.

Lavage may also play a role in the identification of diffuse alveolar haemorrhage (DAH) using iron staining, and in the diagnosis of pulmonary malignant disease or lymphangitis carcinomatosa [7]. Additionally, chemical and functional assays may provide information about noncellular constituents of the lower respiratory tract obtained by BAL [19].

Confounding factors of the BALF profile

Interpretation of data assessed from BALF - distribution of cells as well as fluid constituents - is complicated by factors such as age, smoking history and use of drugs, especially, immunoregulators, as well as the lavage technique [7, 13, 19]. Various technical aspects of BAL are critical in obtaining representative samples.

The epithelial cell layer is extremely vulnerable to trauma. Damage caused by insertion of a fibreoptic bronchoscope into the airways may result in a number of confounding factors including an increased number of neutrophils and erythrocytes in the BALF samples [7, 13, 19]. Smoking has been found to adversely affect the alveolar micro-environment both in health and disease [7, 14]. In the normal population, cigarette smoking has significant effects on BALF samples: the fluid recovery and the viability of the cells decrease, the number of red cells contaminating BALF samples increases, and the quantity of cellular and noncellular constituents changes [3, 4, 12-14, 19].

Diagnostic applications of BALF analysis in patients with diffuse lung disease

The diagnostic evaluation of patients with DILD is a clinical challenge. Thorough clinical assessment should be considered as the key diagnostic procedure as a specific diagnosis, even with lung biopsy, is dependent on clinical information. To establish or confirm the diagnosis in patients with DILD many ancillary noninvasive procedures are required [1]. Although histologically confirmation is preferable and tissue specimen are sometimes mandatory for a definitive diagnosis, BALF analysis offers the opportunity to establish the diagnosis in selected cases. If analysis of BALF is diagnostic or highly suggestive of the underlying disease process with only a low risk of a false diagnosis, it obviates more invasive procedures such as biopsy procedures [1, 2, 9, 14]. In the appropriate clinical setting, the diagnosis of alveolar proteinosis, pulmonary histiocytosis X, extrinsic allergic alveolitis or hypersensitivity pneumonitis and drug-induced pneumonitis, eosinophilic pneumonia, pulmonary haemosiderosis, sarcoidosis and infections may be established with appropriate analysis of BALF [8, 25]. Together with a thorough clinical evaluation BALF analysis may be the key to diagnosis making lung biopsy unnecessary in most cases. However, if despite this thorough clinical evaluation the diagnosis remains unclear, a biopsy should be considered as the final diagnostic step [1, 2]. Prospective studies are needed to address the cost effectiveness of clinical assessment, routine high resolution computed tomography (HRCT), BAL, transbronchial biopsy, surgical lung biopsy (open *versus* thoracoscopic) as diagnostic procedures and their effect on the clinical outcome [1].

Cellular BALF characteristics as valuable adjunct to diagnosis

Table 2. – Normal values of the cellular bronchoalveolar lavage profile		
	Nonsmokers	Smokers
<i>Yield</i>		
mL	138±89(110-170)	128±15(100-150)
percentage	59.3±3.4(55-85)	57.8±2.3(50-75)
<i>Total cell count</i>		
cells x10 ⁴ .mL ⁻¹	10.3±1.6(10.1-18.2)	33.2±3.9(25-60)
<i>Alveolar macrophages</i>		
cells x10 ⁴ .mL ⁻¹	9.3±1.4(9.0-10.5)	27.9±3.2(25-42)
percentage	89.8± 0.7(83-95)	94.5± 0.6(90-95)
<i>Lymphocytes</i>		
cells x10 ⁴ .mL ⁻¹	0.8±0.1(0.7-1.5)	0.8±0.3(0.6-1.5)
percentage	8.4±0.7(7.5-12.5)	3.8 ±0.5 (2.5-12.5)
<i>Polymorphonuclear neutrophils</i>		
cells x10 ⁴ .mL ⁻¹	0.10±0.03(0.05-0.25)	0.31±0.90(0.25-0.95)
percentage	1.3±0.2(1.0-2.0)	1.2±0.2(1.0-2.5)
<i>Eosinophils</i>		
cells x10 ⁴ .mL ⁻¹	0.03±0.05(0.02-0.08)	0.14±0.03(0.10-0.35)
percentage	0.44±0.11(0.21-0.52)	0.43±0.08(0.30-0.80)
<i>Mast Cells</i>		
cells x10 ⁴ .mL ⁻¹	0.01±0.01(0.01-0.02)	0.16±0.06(0.02-0.09)
percentage	0.09±0.03(0.10-0.35)	0.43±0.08(0.02-1.01)
<i>CD4⁺/CD8⁺-ratio</i>	2.6±0.2(2.2-2.8)	1.5±0.1(0.7-1.8)

Data are expressed as mean values±SEM with range in parentheses.

Normally, BALF samples, obtained from healthy nonsmoking controls, contain 80-90% alveolar macrophages (AMs), 5-15% lymphocytes, 1-3% polymorphonuclear neutrophils (PMNs), <1% eosinophils and <1% mast cells [12, 14] (table 2).

The aforementioned cell populations present within the lung are all potentially inflammatory cells. In patients with DILD marked changes in cell yield and cell differentiation may occur (table 3). To date, cellular analysis of the alveolar component reveals essentially no squamous epithelial cells and rare bronchial cells. Therefore, analysis of BALF showing >1% epithelial cells likely contains contamination from bronchial material.

Table 3. - Characteristics of the cellular BALF profile in the most common (diffuse) lung diseases.

	AMs	Lym	PMNs	Eos	PC	MC	CD4/CD8 ratio
Noninfectious diseases							
Sarcoidosis		+	=	=/+	0	=/+	+/-/-
Extrinsic allergic alveolitis	'Foamy' aspect	++	+	=/+	1/0	++	-/=
Drug-induced pneumonitis	'Foamy' aspect	++	+	+	1/0	++	-/=
Idiopathic pulmonary fibrosis		+	+/>++	+	0	+	=
BOOP	'Foamy' aspect	+	+	+	0/1	=/+	-
Eosinophilic pneumonia		+	=	++	1/0	=/+	-
Alveolar proteinosis	'Foamy' aspect	+	=	=	0	?	+/=
Connective-tissue disorders		+	=/+	=/+	0	=/+	+/>-/-
Pneumoconiosis	Inclusion particles	+	+	=/+	0	=/+	+/>-/-
Diffuse alveolar haemorrhage	Fe-staining:+++	=/+	+	=/+	0	?	=
ARDS	Fe-staining:+	+	++	+	0	=/+	-/=
Malignancies							
Bronchus carcinoma		=	=	=	0	=	=
Lymphangitis carcinomatosa		+	+/>=	+/>=	0/1	+/>=	-/=
Hematologic malignancies		+	+	+	0/1	+	-/=
Infectious diseases							
Bacterial	Intracellular bacteria	=	++	+	0	?	=
Viral	Inclusion bodies	+	+	+	0	?	-/=
Tuberculosis		+	=	=/+	0	+	=
HIV infection (AIDS)	Inclusion bodies (viral infections)	+	+	+/>=	0	?	-

?=not known. +, ++: increased;=: normal; -: decreased; 0: not present; 1 present; AMs: alveolar macrophages; Lym: lymphocytes; PMNs: polymorphonuclear neutrophils; Eos: eosinophils; PC: plasma cells; MC: mast cells; BOOP: bronchiolitis obliterans with organizing pneumonia; ARDS: adult respiratory distress syndrome.

Specific diseases

Sarcoidosis

Among other clinical features, sarcoidosis is characterized by a predominant increase of the absolute and relative number of lymphocytes and an increase of the CD4/CD8 ratio in BALF [14-18, 26]. However, other DILD (table 3), as well as other extra thoracic granulomatous diseases, such as Crohn's disease and primary biliary cirrhosis may demonstrate a lymphocyte alveolitis similar to sarcoidosis. Furthermore, diseases with an increased number of lymphocytes in BALF can be further differentiated into those with an elevated, normal, or decreased CD4/CD8 ratio (table 3). However, neither the number of lymphocytes nor the CD4/CD8 ratio in BALF are specific features of any pulmonary disorder [9]. Recently, in a sarcoidosis patient population 18% of the cases were identified with a CD4/CD8 ratio in BALF below 2.0, whereas in a population of patients suffering from extrinsic allergic alveolitis 12% of the cases had a ratio above 3.5 [27], indicating that this ratio has limited diagnostic power. In line with this, KANTROW *et al.* [28] reported that the CD4/CD8 ratio in sarcoidosis appeared to be highly variable.

In contrast, differentiation of cells present in BALF, e.g. AMs, lymphocytes, PMNs and eosinophils was found to be much more disease specific [14, 29]. The clinical significance of BALF cell profiles depends on the extent to which they reflect the underlying pathological process within the lung. A number of selected variables provided from BALF analysis appeared to be helpful in predicting the diagnosis of an arbitrary patient suspected of having one of the three most common DILD (table 4) [14].

Patient one suffered from dry cough, fatigue and chest pain. The chest radiograph demonstrated diffuse interstitial infiltration of both lungs. The cellular BALF analysis results are summarized in table 4. Not a predominant cell type present in BALF appeared to be important, but a combination of features, an elevated total cell count, predominantly lymphocytes, together with a nearly normal percentage of eosinophils and PMNs and the lack of plasma cells, made it possible to distinguish the most likely diagnosis sarcoidosis from extrinsic allergic alveolitis and idiopathic pulmonary fibrosis. Ultimately, the diagnosis was histological confirmed. Therefore, careful evaluation of the cellular profile, together with an extensive evaluation of clinical and radiological features, allows prediction of the underlying disorder with a high sensitivity and specificity [14]. These three disorders, sarcoidosis, extrinsic allergic alveolitis or hypersensitivity pneumonitis, associated either with a drug reaction or inhalation of antigens causing a host response, and idiopathic pulmonary fibrosis, may display similarities in clinical presentation, but demonstrate a different cellular profile in BALF (table 4) [14].

Extrinsic allergic alveolitis or hypersensitivity pneumonitis

The suspicion of a certain diagnosis such as hypersensitivity pneumonitis is generally raised by the elicitation of

a history of exposure of respirable antigens, such as mammalian and avian proteins, the use of certain drugs [30-34], and by altered environmental factors. The presence of plasma cells together with "foamy macrophages" and an increase of the number of lymphocytes in BALF is very suggestive of the diagnosis extrinsic allergic alveolitis or drug-induced hypersensitivity pneumonitis [35-39]. In patient 2 (table 4) the total cell count was increased, predominantly the lymphocytes, but also the PMNs and eosinophils were found to be high. Furthermore, some plasma cells were found. This BALF profile, together with the knowledge that the patient kept parrots, and a reticulonodular pattern demonstrated on a HRCT scan made the diagnosis extrinsic allergic alveolitis, highly likely [14, 35]. Moreover, after avoidance of exposure to the parrots the patient's clinical condition improved and a control HRCT scan showed none of the earlier reported abnormalities anymore. In patient three a rather similar profile was found. This patient was known to have rheumatoid arthritis and had recently been treated with gold injections. Thereafter, she developed a drug-induced pneumonitis induced by gold [39]. After the treatment with gold was discontinued and a short period (two weeks) of corticosteroid treatment was carried out, the clinical condition as well as functional and radiographic features of this patient improved dramatically. Other diseases with presence of plasma cells in BALF include bronchiolitis obliterans with organizing pneumonia (BOOP), chronic eosinophilic pneumonia (CEP), *Legionella* pneumonia and malignant non-Hodgkin's lymphoma [35, 37].

Table 4. - Summary of BALF characteristics of some patients suffering from various diffuse interstitial lung diseases.

	Patient					
	1	2	3	4	5	6
Age, yrs	30	45	48	51	30	58
Sex	male	female	female	male	female	male
Smoking	no	no	no	no	no	no
Yield, mL	128	75	120	80	50	75
TCC x 10 ⁴ .mL ⁻¹	29	110	100	20	64	22
Alveolar macrophages, %	65.8	18.2	19.6	65.7	43.2	6.1
Lymphocytes, %	33.2	61.6	51.0	14.8	43.2	19.9
PMNs, %	0.6	12.8	22.2	12.4	4.2	84.0
Eosinophils, %	0.2	6.2	7.0	6.8	42.8	0
Mast cells, %	0.2	1.0	0.2	0.3	0.4	0
Plasma cells, %	0	0.2	0	0	0	0
CD4/CD8 ratio	1.2	1.8	1.9	2.8	0.8	2.2
Culture	negative	negative	negative	negative	negative	negative
Most likely diagnosis	Sar (99.9%)*	EAA (99.6%)*	DP (98.1%)*	IPF (94.3%)*	AEP (nd)	SwS (nd)

TCC: total cell count; PMNs: polymorphonuclear neutrophils; Sar: sarcoidosis; EAA:extrinsic allergic alveolitis induced by parrots; DP: drug-induced pneumonitis induced by gold in a patient with rheumatoid arthritis; AEP: acute eosinophilic pneumonia induced by minocycline; SwS: Sweet's syndrome with pulmonary involvement associated with myelodysplasia [46]; nd: not done; *most likely diagnosis with likelihood ratio as predicted with a computer model.

Idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis or cryptogenic pulmonary fibrosis has no pathognomic clinical, biochemical or pathologic features. This entity is currently diagnosed by exclusion of other specific entities resembling idiopathic pulmonary fibrosis. In line with this, the lavage profile alone is nonspecific in idiopathic pulmonary fibrosis [12, 14]. However, the cellular BALF profile appeared to be quite different from the BALF profile assessed from patients suffering from disorders with similar clinical presentation, e.g. sarcoidosis or hypersensitivity pneumonitis (table 4, patient 4) [14]. An increase in the number of PMNs in 70-90% of the cases, together with an increase in the number of eosinophils in 40-60%, and, additionally, an increase in lymphocytes in 10-20% was reported [11, 12, 14]. An increase in lymphocytes alone is quite rare in idiopathic pulmonary fibrosis, and other diseases should then thoroughly be excluded. Although BALF cytology is nonspecific, it can be very helpful in certain clinical circumstances. For example, a patient with slowly progressive DILD, finger clubbing, and subpleural fibrosis with honeycombing found on HRCT.

Other diffuse interstitial lung diseases

A pronounced increase of the number of neutrophils is also found in other lung diseases, e.g. acute respiratory distress syndrome (ARDS) [40], bacterial pneumonia [25, 41], subacute extrinsic allergic alveolitis [8, 38], collagen vascular diseases [8, 42], connective-tissue disorders [8, 43] or idiopathic bronchiolitis obliterans [43, 44] (table 3). Furthermore, myeloproliferative disorders associated with Sweet's syndrome may involve extra cutaneous sites including the lung (table 4) [46, 47].

A predominant eosinophilia is indicative of an eosinophilic pneumonia, the Churg-Strauss syndrome, allergic bronchopulmonary aspergillosis, or a drug-induced eosinophilic lung reaction (tables 3,4) [8, 11, 48]. Using monoclonal antibody techniques, pulmonary histiocytosis X can be diagnosed in the appropriate clinical setting

(CD1+ Langerhans cells >4% in 50% of patients) [8, 11].

Mast cells or basophils are found to be increased in BALF obtained from patients suffering from extrinsic allergic alveolitis (hypersensitivity pneumonitis), especially, from those patients suffering from the acute form [37, 49]. In this respect, an involvement of mast cells in the antigen-dependent effector phase of delayed hypersensitivity responses has been implicated. Furthermore, increases in BALF mast cells have been reported in tuberculosis, malignant lymphomas and idiopathic pulmonary fibrosis as well as asthma, and, although to a much lesser extent, in sarcoidosis [50].

Identifying characteristic cells, which normally are not present in BALF specimen, may be helpful in confirming a suspected diagnosis, such as malignant cells, e.g. Reed-Sternberg cells in BALF of a patient with a pulmonary localization of Hodgkin's disease [29] or lymphoplasmocytoid cells in BALF obtained from a patient suffering from pulmonary infiltration associated with myelodysplasia [46].

Increasing numbers of patients who are immunocompromised, either by HIV-infection or by receiving immunosuppressive treatment for malignancy or organ transplantation, are prone to develop pulmonary infections, including *Pneumocystis carinii* pneumonia, cytomegalovirus (CMV), fungal pneumonia, and mycobacterial disease. In this setting, lavage has probably achieved the most potential practical value in identifying such infections and differentiating them from alveolar haemorrhage, pulmonary involvement by the underlying malignancy [8], and drug-induced pneumonitis [11,30-34]. The sensitivity of BALF analysis in the diagnosis of bacterial infections ranges from 60-90%; in mycobacterial, fungal, and most viral infections from 70-80%; and in *P. carinii* pneumonia 95% and higher [9].

In pulmonary alveolar proteinosis, BALF analysis may obviate the need of biopsy in almost all cases. The gross appearance of the fluid is milky and turbid. Light microscopy reveals acellular oval bodies; few and "foamy" macrophages; and a dirty background due to large amounts of amorphous debris [7, 8, 11]. Foamy macrophages or 'lipid-laden' alveolar macrophages might also be identified in BALF samples obtained from patients suffering from hypersensitivity pneumonitis [36, 38], drug-induced pneumonitis [30-35] or lipid pneumonia either caused by injection or inhalation of oil substrates [8, 51, 52].

Diffuse alveolar haemorrhage (DAH) or the alveolar haemorrhage syndromes, associated with disorders such as Goodpasture's syndrome, Wegener's granulomatosis and other vasculitides, idiopathic pulmonary haemosiderosis, collagen vascular diseases and drug reactions, can be established by BAL, even if the bleeding is occult, by identifying numerous haemosiderin-laden macrophages [7, 8]. Diffuse alveolar damage (DAD), a pattern of injury in acute DILD, particularly in patients with adult respiratory distress syndrome (ARDS) and those who are immunosuppressed, also is associated with alveolar haemorrhage syndromes, drugs [53, 54] and toxic inhalants [8]. In patients with fresh bleeding episodes, free red blood cells can be found in BALF as well as fragments of red blood cells in the cytoplasm of AMs [8]. As many syndromes may cause DAH other clinical and laboratory features are required to establish the cause of the bleeding [8].

Noncellular constituents of BALF

Analysis of the soluble components of BALF has gained increasing attention [19]. However, it is impossible to evaluate completely the vast repertoire and clinical application of this potent group of effector molecules. Soluble BALF components may originate from various sources including passive transudation, active transport, and local production [7, 19]. The various inflammatory cells discussed above, which mediate their responses by releasing or generating chemical compounds or by recruiting other cells to release or activate additional inflammatory mediators, account for the local production. The number of cytokines and other biological mediators detected and quantified in the lower respiratory tract continues to increase, but, so far, none has achieved clinical usefulness. The majority of the noncellular components include proteins, such as cytokines and immunoglobulins, surfactant-like lipids and phospholipids, complement factors and enzymes [54]. The quantitative expression of noncellular lavage constituents is hampered by the lack of satisfactory reference standards to correct for the variable and unpredictable dilutional effects of the epithelial lining fluid during the procedure.

Attempts have been made to evaluate the clinical relevance of all these solutes. It is assumed that the most reliable BALF solute data are derived from investigating large molecular weight substances (which are unlikely to move acutely from plasma to BALF) and using comparative ratios of one with another. In extrinsic allergic alveolitis, the immunoglobulin (Ig) levels, as well as the ratios of IgA, IgM, and IgG to albumin, appeared to be increased in BALF compared to plasma. Local synthesis of Igs by plasma cells has been suggested to account for these changes [11, 38]. Identification of paraproteins in BALF samples may be of additional value to distinguish between a pulmonary localization of malignant lymphomas and other pulmonary disorders [46].

For screening the degree of pulmonary damage, the analysis of BALF can be limited to a relatively small number of parameters [55, 56]. An important and well known marker of an inflammatory response in the bronchoalveolar region is the number of PMNs present in BALF. Furthermore, assessing cellular enzymes - such as lactate dehydrogenase (LDH), alkaline phosphatase - appeared to be of additional value indicating which cells are involved in the pathological process [55]. An increase of LDH levels in BALF appeared to be associated with AMs or PMNs involvement [57, 58]. Recently, the LDH isoenzyme pattern was found to be more or less cell specific. A low LDH3/LDH5 ratio was found to be related to AMs, whereas a high ratio was related to PMNs [57, 58]. An increased alkaline phosphatase activity in BALF has been associated with increased secretory activity of cells normally not

present in BALF, e.g. alveolar type II cells [59-61]. An accurate view of the patient at work or in the milieu where respiratory symptoms occur can provide important clues. Dust particles in AMs or elevated asbestos body counts in BALF and/or the presence of birefringent material or inclusion bodies [62], point towards dust or fibre exposure that may cause illness [8, 62]. Additionally, inhalation challenge with the offending substance in a controlled setting to reproduce symptoms, can confirm and/or modify the presumable association of the respiratory symptoms with the exposure.

Conclusion

Bronchoalveolar lavage, an easily performed and well tolerated procedure, is able to provide cellular contents, cellular products, and proteins from the lower respiratory tract. When applied according to standardized protocols, and, considered in the context of other information from conventional ancillary diagnostic tests, BAL appears to be useful in the diagnosis of diffuse lung disease. In this respect, BAL has the advantage of avoiding more invasive diagnostic procedures, such as tissue biopsies, in selected cases. It is likely that BAL will continue to serve as an important procedure for clinical purposes, as well as a method that facilitates the understanding of pathogenesis in processes causing diffuse lung disease.

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A computer program using BALF analysis results as a diagnostic tool in interstitial lung diseases

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Abstract

Background-Recently, we showed that it is possible to distinguish between three common interstitial lung diseases (ILD) with similarities in clinical presentation by using a number of selected variables derived from bronchoalveolar lavage fluid (BALF) analysis. The aim of this study was to develop a more general discriminant model, based on polychotomous logistic regression analysis.

Study design-The 277 patients involved in the study belonged to diagnostic groups with sarcoidosis (n=193), extrinsic allergic alveolitis (EAA; n=39), and idiopathic pulmonary fibrosis (IPF; n=45). The diagnosis had been established independently of the BALF-analysis results. **Results**-The variables used to discriminate among these patient groups were the yield of recovered BALF, total cell count, and percentages of alveolar macrophages, lymphocytes, neutrophils, and eosinophils. In order to test the predictive power of the logistic model, we used 128 patients having sarcoidosis (n=91), EAA (n=5), or IPF (n=32) from another hospital. In this test set the agreement of predicted with actual diagnostic-group membership was the same as in the learning set in which the logistic model was fitted: 94.5% of the cases were correctly classified.

Conclusion-A validated computer program based on the polychotomous logistic regression model can be used to predict the diagnosis for an arbitrary patient with information provided by BALF-analysis, and is thought to be of diagnostic value in patients suspected of having ILD.

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Introduction

Bronchoalveolar lavage (BAL) has the advantage of having a high sensitivity for the diagnosis and evaluation of a great variety of inflammatory processes in the lung, particularly interstitial lung diseases (ILD) [1-4]. Common clinical, radiographic, and pathophysiologic features form the basis for collectively referring to this complex group of disorders as ILD. Despite a thorough history and physical examination, blood and serologic tests, imaging procedures and physiologic tests, a specific diagnosis most often requires the use of specialized procedures such as fiberoptic bronchoscopy with BAL, transbronchial biopsy, and thoracoscopic or surgical lung biopsy [2]. Since BAL, a safe and minimally invasive procedure, enables cellular and noncellular components from small airways and alveolar spaces to be obtained and examined, it may replace more invasive diagnostic procedures in the evaluation of ILD. In addition, many studies employing BAL have resulted in major advances in understanding of the cell biology of the lung and of the pathogenesis of many pulmonary diseases [5-7]. Usually, ILD show an alveolitis characterized by an accumulation of inflammatory and immune effector cells within the interstitium and the alveolar spaces. The type of inflammatory cells in the lower respiratory tract may vary among the various ILD, and helps to confirm the diagnosis [1,3,6,8]. Excess activated, proliferating T-lymphocytes in the BAL fluid (BALF) are associated with granulomatous diseases such as sarcoidosis and hypersensitivity reactions such as extrinsic allergic alveolitis (EAA) [9-11]. However, the composition of lymphocyte subpopulations in BALF differs in these disorders. EAA is mainly characterized by a low CD4⁺/CD8⁺ (helper:suppressor) ratio [9,10]. In contrast, a high CD4⁺/CD8⁺ ratio is frequently found in sarcoidosis [1,6]. Also, in EAA, a mild increase in polymorphonuclear neutrophils (PMNs) and mast cells can be found shortly after the inhalation of antigen [8,12,13]. Moreover, the presence of plasma cells in BALF samples is highly suggestive of EAA [14]. Despite random increases in BALF lymphocytes, PMNs, and eosinophils in about two-thirds of IPF patients, there are no typical cellular BALF features of this disease [1,15,16].

The predominant inflammatory cells obtained by BAL therefore provide a useful indication of the nature of the

underlying disease, particularly when the diagnosis is not clear from other investigations. Moreover, because biopsy specimens cannot always be obtained, it is sometimes necessary to do without histologic confirmation. Therefore, BAL appears to be a useful adjunct to clinical findings and findings in other investigations. However, until now, there has been no standardized consensus about features of the cellular composition of BALF in the respective pulmonary disorders.

In a previous study [1], we showed that it is possible to distinguish between the three most common kinds of ILD in case with similarities in clinical presentation (*i.e.*, sarcoidosis, EAA and IPF, which collectively constitute about 90% of cases of ILD). Besides the yield of recovered BALF, selected cellular variables derived from BAL analysis that could be used to discriminate among the three diagnostic groups were the total cell count and the percentages of alveolar macrophages, lymphocytes, PMNs, and eosinophils.

The purpose of this study was try to standardize the diagnostic workup of patients with ILD using the results of BALF analysis, with sarcoidosis, EAA, and IPF as examples.

We especially wanted to show how improvements can be made in the approach to analyzing BALF results, and to achieve greater efficiency in interpreting these results, so as to make BALF analysis more universal and independent of individual investigator interpretation and therefore, more valuable in patients with ILD. In view of this, we developed a computer program based on polychotomous logistic regression analysis. In order to evaluate the predictive power of this program, we tested it in a patient population with ILD from another hospital.

Methods

Patients

Retrospectively, the initial BALF specimens of patients with sarcoidosis, EAA and IPF (n=277) were selected from all BALF analyses (n=2,008) performed during the 10-yr period, from 1980 to 1990 in the Sint Antonius Hospital, Nieuwegein, the Netherlands. This earlier study included consecutive patients with sarcoidosis (n=193) at the time of diagnosis. The patient group consisted of patients with disease detected on a routine chest radiograph (n=37), patients with respiratory and general constitutional symptoms (n=110), and patients with erythema nodosum and/or arthralgia and hilar lymphadenopathy (*i.e.*, Löfgren's syndrome; n=46). All patients presented with Stage I or II disease by chest radiography; none presented with Stage III disease. The diagnosis was histologically proven by biopsy of mediastinal lymph nodes, transbronchial biopsy, open-lung biopsy, or liver biopsy. The BAL used in the present study was done when the sarcoidosis patients were admitted to the hospital to establish the diagnosis of their disease and before corticosteroids were given.

The diagnosis of EAA was based on clinical information, chest radiography, pulmonary function tests, the presence of precipitins in peripheral blood, and the disappearance of symptoms after avoidance of the causative antigen or, in some cases, after a short course of treatment with corticosteroids. All EAA patients (n=39) had had recent contact with the causative antigen, but not within the last 48 h before BAL.

Patients with IPF (n=45) commonly presented with an onset of breathlessness with exercise and a nonproductive cough, and sometimes with constitutional symptoms. The diagnosis IPF was based on clinical information, chest radiography, pulmonary function tests (*i.e.*, a decreased lung compliance and diffusion capacity for carbon monoxide; and hypoxemia, especially with exercise, without hypercapnia). Additionally, IPF was histologically proven by open-lung biopsy. The demonstrated alveolitis was characterized by an infiltration of mononuclear cells, interstitial pneumonitis, and/or derangement of parenchymal structures (*i.e.*, fibrosis). No patient received corticosteroids or other medications, either at the time of lavage or before. The control group consisted of 30 healthy volunteers, without any history of pulmonary disease, who had normal chest radiographs and lung function tests.

Groups	n	m*	Age	Female	Male	NSm	Sm
Controls	30	0	33 (21-55)	15	15	15	15 (14.9±8.8)
<i>Learning set</i>							
Sar	193	3	35 (18-79)	96	94	145	45 (15.7±7.8)
EAA	39	1	50 (23-78)	14	24	34	4 (12.8±6.9)
IPF	45	1	60 (30-79)	16	28	27	17 (18.0±5.1)
<i>Test set</i>							
Sar	91	0	37 (17-77)	55	36	79	12 (15.7±6.1)
EAA	5	0	52 (40-66)	1	4	3	2 (20.0±5.0)
IPF	32	0	66 (50-79)	6	26	21	11 (17.4±8.1)

Definition of abbreviations: m=missings, *missing at least one discriminating variable and thus not used in analysis; Mean with range in parentheses; NSm=Nonsmokers; Sm=Smokers; Number of smokers, mean number of cigarettes a day ± standard deviation in parentheses; Sar=sarcoidosis; EAA=extrinsic allergic alveolitis; IPF=idiopathic pulmonary fibrosis.

In order to test the predictive power of the computer program based on the logistic model fitted in the learning set described previously, we used a set of 128 patients from another hospital (*i.e.*, the Rijnstate Hospital, Arnhem, the Netherlands) who had sarcoidosis, EAA, or IPF. The diagnostic procedures used for this test population were comparable with those for the population that we used to develop the computer program (the learning set). In summary, the diagnoses of sarcoidosis and IPF were proven histologically by biopsy, and the diagnosis of EAA was confirmed by clinical information, chest radiography, pulmonary function testing, the presence of precipitins in peripheral blood, and the disappearance of the symptoms after avoidance of the causative antigen, or, in some cases, after a short course of treatment with corticosteroids. Table 1 lists the characteristics of the control subjects and patient groups studies. The study was approved by the ethical committees of both participating hospitals.

Bronchoalveolar lavage

As reported previously [1], BAL was performed during fiberoptic bronchoscopy. Because the BAL procedure was the same in both hospitals, the results were fully comparable. The patients were premedicated with atropine and sometimes diazepam or codeine, and given local anaesthesia of the larynx and bronchial tree (tetracaine 0.5%). BAL was performed by standardized washing of the middle lobe with four 50-mL aliquots of sterile saline (0.9% NaCl) at 37°C. Peripheral blood samples were taken simultaneously.

Recovered BALF was kept on ice in a siliconized specimen trap, and was separated from cellular components by centrifugation (5 min, at 350 x g). Supernatants were directly stored at -70 °C after an additional centrifugation step (10 min, at 1,000 x g). Cells were washed twice, counted, and suspended in minimal essential medium (MEM; Gibco, Grand Island, NY) supplemented with 1% bovine serum albumin (BSA; Organon Teknika, Boxtel, the Netherlands). Preparations of cell suspensions were made in a cytocentrifuge (Shandon, Runcorn, UK). Cytospin slides of BALF cells were stained with May-Grünwald-Giemsa (MGG; Merck, Darmstadt, Germany) for cell differentiation. At least, 1,000 cells were counted.

Statistical methods

In order to distinguish the three diagnostic groups from one another, a polychotomous logistic regression analysis [17,18] was done on the learning set, according to the following procedure: Each of the 277 patients in the learning set belonged to one and only one diagnostic group. Five of the cases (three sarcoidosis patients, one EAA patient, and one IPF patient) had at least one missing discriminating variable, and 272 cases were therefore used for the analysis. Of these, 190 patients belonged to the sarcoidosis group, 38 to the EAA group, and 44 to the IPF group (Table 1). Hence, an arbitrary patient within the total learning set had a probability of $190/272=0.70$, $38/272=0.14$

and 44/272=0.16, respectively, of belonging to each of the three diagnostic groups. These probabilities, which add up to 1 (as they should), are called "prior probabilities". If a set of predefined characteristics (so-called "explanatory variables") of a patient is known, these characteristics can be involved in varying the prior probabilities for that particular patients. For example, the probabilities for a patient who is a smoker may differ from the aforementioned probabilities, and also from the probabilities of a nonsmoker. The latter probabilities, which can be calculated if we know the smoking status of a patient, are the so-called "posterior probabilities". Polychotomous logistic regression analysis is a statistical technique that can be used to calculate these posterior probabilities from the prior probabilities and from the patient characteristics. If there are only two diagnostic groups, the analysis is simply called a logistic regression analysis, without the adjective "polychotomous".

By means of polychotomous logistic regression analysis, an allocation rule can be derived according to which an arbitrary patient is allocated to one and only one of J disjoint diagnostic groups, $j = 1, \dots, J$, on the basis of p explanatory variables X_1, X_2, \dots, X_p , that contain some information about the diagnostic group to which the patient belongs. This information is assembled in J - 1 linear predictor scores Y_j , which are defined as linear combinations of the explanatory variables:

$$Y_j = \beta_{0j} + \beta_{1j}X_1 + \dots + \beta_{pj}X_p, \quad j=1, \dots, J-1 \quad (1)$$

Hence, there are J - 1 sets of unknown coefficients $\{\beta_{0j}, \beta_{1j}, \dots, \beta_{pj}\}$ to be estimated. For this purpose a polychotomous logistic regression model is postulated for the probability p_j , defined as the probability that a patient with scores y_1, \dots, y_{J-1} belongs to a particular diagnostic category j, as follows:

$$p_j = \frac{\exp(y_j)}{1 + \sum_{j=1}^{J-1} \exp(y_j)} \quad (2)$$

For $j = 1, \dots, J - 1$,

$$p^J = \frac{1}{1 + \sum_{j=1}^{J-1} \exp(y_j)} \quad (3)$$

with $\sum_j p_j = 1$.

The coefficients $\{\beta_{0j}, \beta_{1j}, \dots, \beta_{pj}\}$ in the linear predictor scores y_j ($j=1, \dots, J-1$) can be estimated by means of the maximum likelihood technique, using standard statistical software (e.g., the polychotomous logistic regression [PR] module of the Biomedical Package [BMDP]). After estimating these coefficients, the probability p_j can be calculated for each patient with the foregoing formula, using that patient's values of the explanatory variables, x_1, \dots, x_p . The value of j for which p_j is maximum for a patient is defined as the predicted group to which the patient belongs. When the β coefficients are estimated from the total number, n, of patients, with n_j patients per diagnostic category j, then an

additive part in the estimated $\hat{\beta}_{0j}$ coefficients is $\ln(n_j/n_J)$:

$$\hat{\beta}_{0j} = \hat{a}_j + \ln(n_j/n_J), \quad j = 1, \dots, J-1, \quad (4)$$

which represents the only coefficients influenced by the relative sizes of the diagnostic groups. For predicting the group memberships of patients in a population whose diagnostic group have other relative sizes (e.g., N_j/N_{j0}),

then $\hat{\beta}_{0j}$ coefficients have to be adapted as follows:

$$\hat{\beta}_{0j(\text{new})} = \hat{\beta}_{0j} - \ln(n_j/n_J) + \ln(N_j/N_J) \quad (5)$$

In our application with three diagnostic groups ($J = 3$), we had two linear predictors, of which the estimated β -coefficients and standard errors are presented in Table 2.

Table 2.- Estimated coefficients and standard errors (SE) of the polychotomous logistic regression model.

Explanatory variable	F1	SE	F2	SE
Constant	11.68	4.26	16.19	5.51
Age yr	-0.2072	0.05	-0.1406	0.05
Sex (female)	0.7863	0.82	0.9682	1.01
Smoking (yes)	2.389	2.65	-0.9572	3.15
Smoking x (age ²)	-0.4481x10 ⁻³	0.82x10 ⁻³	0.8495x10 ⁻³	0.11x10 ⁻²
<i>BALF</i>				
Yield(out/in)x100	0.0672	0.04	-0.0310	0.04
Cells x10 ⁴ .mL ⁻¹	-0.0764	0.02	-0.0120	0.02
AMs %; squared	-0.2913x10 ⁻³	0.41x10 ⁻³	-0.2009x10 ⁻²	0.82x10 ⁻³
PMNs %; squared	0.5536x10 ⁻³	0.55x10 ⁻³	-0.2613x10 ⁻²	0.22x10 ⁻²
Lym %; squared	0.1465x10 ⁻²	0.13x10 ⁻²	0.6466x10 ⁻³	0.15x10 ⁻²
Eos %; squared	-0.1986	0.0623	-0.0109	0.0116

Definition of abbreviations: F1=function 1; F2=function 2; AMs=alveolar macrophages; PMNs=polymorphonuclear neutrophils; Lym=lymphocytes; Eos=eosinophils.

After the logistic regression analysis was done, a computer program was created according to the regression model. This program was tested by predicting ILD, using the population mentioned earlier, that had one of the three ILD from another hospital, that we investigated.

Results

Table 3 contains the results of BALF-cell analyses of the control subjects and patients with sarcoidosis, EAA, or IPF. Since statistically significant differences were found among nonsmokers (NSm) and smokers (Sm) within the studied groups, these data are shown separately. The data are presented as mean values \pm SEM.

The classification results for both the learning set and the test set are listed in Table 4. These results were obtained by applying the allocation rule as described earlier. A polychotomous logistic regression analysis was performed on the data for the ILD patients. In this group of 277 patients (the learning set), the computer prediction resulted in a different outcome (*i.e.*, the highest likelihood for 24 patients of a particular diagnosis) than did physicians' judgment based on anamnesis and various tests (mainly biopsy; see Table 4).

The rate of accuracy of classification was independent of the type of ILD, and no patient was excluded because of outlying parameters. Moreover, when the outcome of the program differed from the physician's judgment, the program expressed doubt, and in most cases calculated a substantial percentage, although not the highest, toward the correct diagnosis. Thereafter, in order to test the predictive power of the model, the patient population from the other hospital in the study was used. In the latter group of 128 patients (test set), the computer prediction gave a different outcome for seven patients than did physicians' judgment (Table 4).

The main finding in those cases not accurately categorized by the polychotomous logistic regression analysis (*i.e.*, "diagnostic misses" was a significantly different yield of the BALF from that of the prespective populations in 16 of the 24 misclassified cases in the learning set and five of the seven misclassified cases in the test set (data not shown).

Table 3.-BALF variables from the study subjects used in the polychotomous regression model*.

Study group	Yield	TCCx10 ⁶	AMs	PMNs	Lym	Eos
C (NSm)	65.4±2.2	13.3±1.5	87.0±1.9	1.6±0.4	11.0±1.3	0.34±0.1
C (Sm)	63.2±2.8	30.8±4.9	91.6±2.2	1.2±0.4	6.8±2.4	0.41±0.1
Sar (NSm)	55.6±1.0	21.6±1.8	63.2±1.5	1.7±0.4	34.3±1.5	0.55±0.1
Sar [§] (NSm)	55.9±1.0	22.4±1.4	58.2±2.6	1.8±0.3	39.1±2.5	0.79±0.1
Sar(Sm)	56.6±1.6	23.0±1.8	74.9±2.5	1.2±0.2	23.0±1.8	0.72±0.2
Sar [§] (Sm)	56.9±3.1	25.4±4.7	78.8±4.2	1.2±0.3	19.5±4.6	0.51±0.9
EAA(NSm)	46.4±1.8	41.2±5.0	38.2±1.5	4.3±0.6	58.1±2.6	2.61±0.5
EAA [§] (NSm)	45.8±5.5	33.6±6.2	44.7±5.3	1.1±0.2	51.5±6.1	2.70±2.3
EAA(Sm)	43.4±4.6	50.3±20.6	57.3±5.6	5.8±1.2	40.8± 7.5	3.33±2.3
EAA [§] (Sm)	51.6±11.6	30.4±10.7	35.7±7.8	7.7±4.6	53.6±14.1	2.51±1.2
IPF(NSm)	42.6±2.4	20.9±5.0	67.8±3.7	11.7±1.9	13.8±3.0	6.44±1.8
IPF [§] (NSm)	49.1±1.7	22.7±3.4	72.2±5.1	7.4 ±1.4	11.8±2.1	8.51±3.4
IPF(Sm)	46.1±4.2	29.7±6.2	63.3±8.5	18.9±8.9	10.2±2.7	7.60±2.8
IPF [§] (Sm)	48.4±2.7	46.2±5.8	76.0±6.2	11.5±5.2	5.1±1.2	7.13±2.9

Definition of abbreviations: TCC=total cell count; AMs=alveolar macrophages; PMNs=polymorphonuclear neutrophils; Lym=lymphocytes; Eos=eosinophils; C=control subjects; Sar=sarcoidosis; EAA=extrinsic allergic alveolitis; IPF=idiopathic pulmonary fibrosis; NSm=nonsmokers; Sm=smokers.
 * Data of the studied populations are expressed as mean ± SEM. Percentages of the total cell count (TCC).
 Learning set. [§] Test set.

Table 4.- Classification results for patients with interstitial lung diseases.

Actual Group	Predicted Group Membership (n)				Percentage			
	Sar	EAA	IPF	Total	SPEC	DE	PV ⁺	PV ⁻
<i>Learning set</i>								
Sar	180	4	6	190	85.4	94.7	93.8	87.5
EAA	6	31	1	38	97.9	81.6	86.1	97.0
IPF	6	1	37	44	96.9	84.1	84.1	96.9
Total	192	36	44	272				
<i>Test set</i>								
Sar	85	1	5	91	97.3	93.4	98.8	85.7
EAA	1	4	0	5	99.2	80.0	80.0	99.2
IPF	0	0	32	32	94.8	100	86.5	100
Total	86	5	37	128				

Definition of abbreviations: Sar=sarcoidosis; EAA=extrinsic allergic alveolitis; IPF=idiopathic pulmonary fibrosis; SPEC=specificity; DE=diagnostic effectiveness (*i.e.*, sensitivity); PV⁺ =positive predicted value; PV⁻ =negative predicted value.

Learning set

The three respective prior probabilities that an arbitrary patient with IDL (without using any additional information) actually belonged to a diagnostic group were, for sarcoidosis $100(190/272)=69.9\%$, for EAA $100(38/272)=14.0\%$, and for IPF $100(44/272)=16.2\%$. The percentage of patients correctly classified among all patients with a given actual diagnosis, called the "diagnostic effectiveness" [19] or sensitivity, was $100([180+31+37]/272)=91.2$. The diagnostic effectiveness for sarcoidosis was $100(180/190)=94.7\%$, for EAA $100(31/38)=81.6\%$, and for IPF $100(37/44)=84.1\%$ (Table 4). The predicted value of a classification can be calculated as the probability that a patient actually belongs in the predicted group. For the classification of sarcoidosis, the predicted value (PV⁺) was $100(180/192)=93.8\%$, and for the classification of EAA and IPF, the corresponding values were $100(31/36)=86.1\%$ and $100(37/44)=84.1\%$, respectively.

The specificity, or probability of nonsarcoidosis in the group without sarcoidosis was $100([31+1+1+37]/[38+44])=85.4\%$, for non-EAA it was $100x([180+6+6+37]/[190+44])=97.9\%$, and for non-IPF it was $100([180+4+6+31]/[190+38])=96.9\%$.

The predicted value of the negative result (PV⁻) for the group with nonsarcoidosis was $100([31+1+1+37]/[36+44])=87.5\%$, for non-EAA it was $100([180+6+6+37]/[192+44])=97.0\%$, and for non-IPF it was $100([180+4+6+31]/[192+36])=96.9\%$ (see Table 4).

Test set

In order to validate the computer program in a patient population independent of the learning population, a population having the selected ILD from another hospital was used as a test set. The diagnoses of sarcoidosis, EAA, and IPF had been established by the diagnostic procedures described earlier, independently of the BALF-analysis results.

The three respective prior probabilities that an arbitrary patient (without using any additional information) actually belonged to a diagnostic group were, for sarcoidosis $100(91/128)=71.1\%$, for EAA $100(5/128)=3.9\%$, and for IPF $100(32/128)=25.0\%$.

The percentage of patients correctly classified among all patients in the test set with a given actual diagnosis, or diagnostic effectiveness, was $100([85+4+32]/128)=94.5$. The diagnostic effectiveness for sarcoidosis was $100(85/91)=93.4\%$, for EAA $100(4/5)=80.0\%$, and for IPF $100(32/32)=100\%$ (Table 4). For the diagnosis of sarcoidosis, the predicted value (PV⁺) was $100(85/86)=98.8\%$, and for the diagnoses of EAA and IPF, these values were $100(4/5)=80.0\%$ and $100(32/37)=86.5\%$, respectively.

The specificity, or the probability of the prediction of nonsarcoidosis in the group without sarcoidosis, was $100([4+0+0+32]/[5+32])=97.3\%$, for non-EAA it was $100([85+5+0+32]/[91+32])=99.2\%$, and for non-IPF $100([85+1+1+4]/[91+5])=94.8\%$. The predicted value of the negative result (PV⁻) for the group with nonsarcoidosis was $100([4+0+0+32]/[5+37])=85.7\%$, for non-EAA it was $100([85+5+0+32]/[86+37])=99.2\%$, and for non-IPF it was $100([85+1+1+4]/[86+5])=100\%$ (Table 4).

Discussion

The present study illustrates that the polychotomous logistic regression model (computer program) developed in the study can be used to predict the most likely of the three common diagnoses within ILD (*i.e.*, sarcoidosis, EAA, and IPF), given a patient's cellular BALF-analysis results. The model provided a correct prediction in 91.2% of the learning population, and of 94.5% in the respective independent test set used to validate the computer program, irrespective of any other clinical information. In most cases the program predicted the correct diagnosis with great sensitivity as well as specificity. Moreover, when the outcome of the program differed from the physician's judgment, it calculated a substantial percentage although not the highest, of the correct diagnosis. As far as we know, this is the first validated computer program to be a practical diagnostic guide when interpreting BALF-analysis results of patients with ILD. However, the analysis developed in the present study includes only BALF features having to do with the presentation of the disease; the computer program based on the analysis cannot provide the prognosis for such patients or predict their response of therapy. Furthermore, the program differentiates only the three types of ILD, that were studied, and not among other pulmonary disorders. Disorders with a similar clinical presentation requiring rapid diagnosis and a totally different management approach, such as malignancies and infections, should particularly be distinguished from one another. However, such disorders have specific diagnostic features, such as tumour cells or pathogenic microorganisms, and their diagnosis is not based on inflammatory cell differentiation *per*

se. In view of this, the analysis of BALF should also focus on the presence of malignant cells [20], and BALF should be evaluated by microbiologists as well.

Many factors, related to the BAL procedure and the population being studied are thought to greatly influence both the yield and the profile of BALF. In order to make the procedure as comparable as possible for various pulmonary disorders, we used a standard BAL procedure. The model is adjusted for yield because the recovery is related to the BAL procedure and to pulmonary function, among other factors. Previously, many investigators reported that smoking was a very important confounding factor in BALF analysis [1,3,21-23]. It has been suggested that smoking modifies the inflammatory reaction in the lungs, especially the cellular immunoregulation, and may therefore influence the composition of BALF [21,22]. To this end, we added the smoking status of a patient to the model as a personal characteristic. In using the model, it is therefore of great importance to have correct information about the smoking history of a patient and the patient's environmental exposure.

In this study, all patients who were treated with corticosteroids, which are known immunoregulators [24-26], as well as with any other drugs, that might influence the cellular profile of the BALF, such as immunosuppressive agents, were excluded. When testing the computer program based on the model, we found that the use of corticosteroids at the time of or shortly before BAL was performed was associated with changes in the BALF profile and therefore rendered unreliable the prediction made by the program. We suggest, as have others [26], that one of the most important effects of corticosteroids is their influence on eosinophils. Corticosteroids lead to decreased numbers of eosinophils [25] and downregulation of the function of eosinophils (adherence as well as chemotaxis) [26]. Moreover, many other effects of corticosteroids, such as a downregulation of the function of alveolar macrophages (AMs) and changes in the BALF profile have also been reported [25]. Therefore, when interpreting BALF analysis results such as diagnostic indicators, the importance of knowing whether the patient uses any "confounding" drug should be clear.

Interestingly, including only the BALF cell profile in our model led to a correctly predicted diagnosis in a high percentage of cases. With regard to characteristic changes in the cellular composition of BALF in the selected ILD, our results confirm those described by others, showing high absolute and relative numbers of lymphocytes in sarcoidosis and EAA [6,10,13], high numbers of plasma cells and mast cells in EAA [8,9], and an increased number of PMNs and eosinophils in IPF [3,15,16], in both the learning and test populations. Since practically no BALF plasma cells were found in sarcoidosis or IPF, both of which, as stated earlier, may be difficult to differentiate from EAA, the presence of plasma cells in BALF is highly suggestive of EAA. Because they were an almost "perfect" discriminator, we could not include plasma cells in the logistic regression, and they were not incorporated in our analysis.

WINTERBAUER and coworkers [6] found that a high $CD4^+/CD8^+$ ratio and a low percentage of PMNs and eosinophils in BALF had a positive predictive value in distinguishing sarcoidosis from nonsarcoidosis. Despite this, the inclusion of the BALF $CD4^+/CD8^+$ ratio in our analysis did not result in a better prediction. Furthermore, almost 20% of the patients with sarcoidosis demonstrated a decreased $CD4^+/CD8^+$ ratio, and 10% of the patients with EAA had a $CD4^+/CD8^+$ ratio greater than 4 (data not shown). In these cases, therefore, the $CD4^+/CD8^+$ ratio was not a reliable discriminator of sarcoidosis from EAA. Moreover, inclusion of the BALF yield and cellular variables in these particular only revealed a very high percentage of cases that were already correctly predicted. Consequently, an incorrect prediction based on interpretation of the $CD4^+/CD8^+$ ratio in BALF was avoided. This suggests that the computer program demonstrated in the present study also has a high predictive power among more or less "atypical" BALF-analysis results.

In conclusion, the validated polychotomous logistic regression model provides a very reliable prediction of the correct diagnosis for an arbitrary patient with interstitial lung diseases, given information obtained from BALF analysis. We therefore suggest that the computer developed from the model may improve the diagnostic efficiency of BALF analysis in patients suspected of having one of the three most common types of interstitial lung diseases.

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Computer program supporting the diagnostic accuracy of cellular BALF analysis: a new release

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Abstract

Background - Recently, we developed a validated computer program based on polychotomous logistic regression analysis using bronchoalveolar lavage fluid (BALF) results to distinguish between the three most common interstitial lung diseases (ILD): sarcoidosis, idiopathic pulmonary fibrosis (IPF) and extrinsic allergic alveolitis (EAA) or drug-induced pneumonitis. One of the limitations of this program was that it was not useful in discriminating between infectious disorders and non-infectious disorders.

Study design - Therefore, we added BALF samples obtained from patients with a confirmed bacterial pulmonary infection based on culture results $\geq 10^4$ cfu.ml⁻¹ (Group I: $n=31$) to the study population mentioned above (Group II: $n=272$).

Results - Notably, just one variable, *i.e.* the percentage polymorphonuclear neutrophils, allowed to distinguish between infectious and non-infectious disorders. The agreement of predicted with the actual diagnostic group membership was 99.67% (Group I and Group II). Additionally, 91.2% of the cases with ILD were correctly classified.

Conclusion - This updated windows version 2000 of the validated computer program provides a very reliable prediction of the correct diagnosis for an arbitrary patient with suspected pneumonia or with ILD given information obtained from BALF analysis results, and is thought to improve the diagnostic power of BALF analysis.

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Introduction

Diffuse interstitial lung disease (DILD) poses a significant challenge for the clinician because the aetiology is often unknown [1,2]. To establish the diagnosis a thorough history is essential as it may identify a potential etiologic factor. Many diagnostic procedures are useful, in particular a high-resolution computed tomography (HRCT) scan and a bronchoalveolar lavage (BAL) [1]. A BAL readily explores large areas of the alveolar compartment providing cells, as well as noncellular constituents from the lower respiratory tract. Therefore, BAL represents an additional tool in assessing diseases involving the lung parenchyma. After the introduction as a research tool, BAL has been appreciated extensively for clinical applications [3,4]. When applied according to standardized protocols, and, considered in the context of other information from conventional ancillary diagnostic tests, BAL appears to be useful in the diagnosis of DILD, pneumonia, especially opportunistic infections, and, sometimes malignancies with pulmonary localisation [3-7]. Careful analysis of the cellular BAL fluid (BALF) profile, together with a thorough evaluation of clinical and radiological features, allows prediction of the underlying disorder or ruling out a diagnosis with a high sensitivity and specificity [4]. In this respect, it has the advantage of avoiding more invasive diagnostic procedures, such as tissue biopsies.

If the clinician decides that a BAL might be helpful to provide diagnostic material it is mandatory to have reliable diagnostic criteria. Therefore, the interpretation of cellular BALF analysis results has to be standardized to improve the diagnostic power. Previously, we studied cellular BALF data from a large cohort of patients suffering from either sarcoidosis, extrinsic allergic alveolitis (EAA) or idiopathic pulmonary fibrosis (IPF). A logistic regression equation was constructed, using several BALF parameters as variables to provide the most likely diagnosis [8,9]. The diagnosis had been established independently of the BALF-analysis results. The variables used to discriminate among these patient groups were the yield of recovered BALF, total cell count, and percentages of alveolar macrophages, lymphocytes, neutrophils, and eosinophils. This equation appeared to be accurate in 91.2% of the cases. To date, inclusion of the BALF CD4/CD8 ratio in the analysis did not result in a better prediction. However, one of the limitations of this logistic regression equation was - among others - that it was not useful to distinguish disorders of infectious aetiology and non-infectious aetiology [8,9]. Furthermore, JACOBS *et al.* demonstrated the value of BALF cytological findings for the diagnosis of non-infectious conditions in patients with suspected pneumonia [7]. In line with this, COBBEN *et al.* found that only one cellular variable, the number of polymorphonuclear

neutrophils (PMNs), appeared to distinguish between infectious and non-infectious disorders [10]. Moreover, the first DOS edition of the computer model [9] appeared not to be millennium proof.

Therefore, the aim of this study was to provide an updated clinical useful computer program (a millennium proof windows version) to distinguish the three most common above mentioned ILD, as well as to distinguish those non-infectious disorders from bacterial infectious pulmonary disorders using just one simple software program.

Material and methods

General experimental design

Group I consists of the initial BALF specimens of patients with a confirmed nosocomial bacterial pulmonary infection ($n=33$) selected from BALF analyses performed during a 2-yr period in the University Hospital Maastricht, the Netherlands as previously reported [10]. All these patients were suspected of having pneumonia as defined by clinical and radiological criteria with new, persistent or progressive infiltrate on the chest radiograph [11]. 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* ($n=1$), *Klebsiella oxytoca* ($n=1$), *Citrobacter diversus* ($n=1$), *Pseudomonas aeruginosa* and *Proteus mirabilis* ($n=1$), *Staphylococcus aureus* and *Haemophilus influenzae* ($n=1$), *Streptococcus pneumoniae* and *Neisseria meningitidis* ($n=1$), *Escherichia coli* and *Klebsiella oxytoca* ($n=1$), *Escherichia coli* and *Haemophilus influenzae* ($n=1$), *Proteus mirabilis* and *Haemophilus influenzae* ($n=1$).

Group II consists of the initial BALF specimens obtained from patients with sarcoidosis, EAA or IPF ($n=272$) selected from BALF analyses performed in a 10-yr period at the Sint Antonius Hospital, Nieuwegein, the Netherlands [8]. As previously reported [8,9], the diagnosis sarcoidosis was histological proven. The diagnosis EAA was based on clinical information, chest radiography, pulmonary function tests, the presence of precipitines in peripheral blood and disappearance of symptoms after avoidance of the causative antigen or, in some cases, after a short treatment with corticosteroids. The diagnosis IPF was histological proven. The alveolitis demonstrated an infiltration of mononuclear cells, interstitial pneumonitis and/or derangement of parenchymal structures, *i.e.* fibrosis [8,9].

Measurements

BAL was performed during fiberoptic bronchoscopy. The procedure was reported previously [8-10]. Briefly, BAL was performed by standardized washing of the involved lobe (Group I) or the middle lobe or lingula (Group II) with four aliquots of 50 ml sterile saline (0.9 % NaCl) at 37°C after premedication (0.5 mg atropine intramuscular and sometimes 5-10 mg diazepam orally), and local anaesthesia of the larynx and bronchial tree (lidocaine 0.5%). In Group I, if the patient was intubated, the bronchoscope was introduced through the tube.

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 cytologic and microbiological analysis. The total cell count was performed in a Fuchs-Rosenthal haemocytometer chamber. Cyto-centrifugation was done with the Shandon Cytospin 3 apparatus (Shandon Scientific Ltd. Astmoor, England), using the following conditions; speed 650 rpm, time: 10 min, and acceleration rate: low. 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 staining methods. At least 500 nucleated cells were counted. In Group II infection was excluded based on culture results of BALF. In Group I the number of cells containing intracellular organisms was expressed as a percentage of all nucleated cells counted [12]. 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, as well as BALF samples with *Pneumocystis carinii* cysts or *Mycobacterium tuberculosis*. Quantitative bacterial cultures were performed on appropriate media incubated both aerobically and anaerobically. Mycobacterial and fungal cultures were performed on all BALF samples. Of infectious aetiology, BALF samples were defined as those samples with a quantitative culture yielding 10^4 colony forming units cfu.mL^{-1} .

Statistical evaluation

Logistic regression analysis was used to discriminate Group I ($n=31$; subjects with a pulmonary infection [10]) from Group II ($n=272$; subjects with one of the three most common ILD mentioned above [8,9]). In an earlier study a logistic regression model for distinguishing the three common ILD from each other (sarcoidosis, IPF, and EAA) was developed, using mainly variables derived from BALF. This previously developed logistic regression model had also been tested (validated) in another data set [9].

Rather than considering Group I as a fourth disease and redeveloping and revalidating a new logistic regression model for simultaneously distinguishing four lung diseases from each other, a stepwise strategy is chosen. In the first step a logistic regression model (to be developed in this study) will be used for distinguishing BALF samples obtained from patients with a pulmonary infection (Group I) from BALF of patients with ILD of non-infectious origin

(Group II). If the distinction turns out to be in favour of non-infectious lung disease, in the second step, the already validated logistic regression model can be used for distinguishing between the three non-infectious ILDs: sarcoidosis, IPF and EAA [9].

Results

The characteristics and BALF analysis results of the Group I (10) and Group II [8,9] were reported previously. These data are summarized in table 1. In contrast to what was found in the subpopulations of Group II, in Group I no significant difference was demonstrated between smokers and non-smokers [10].

Study groups (n)	Yield,%	TCCx10 ⁴ .mL ⁻¹	AMs*	PMNs*	Lym*	Eos*
Sar (Nsm) (145)	55.6±1.0 (20.0-85.0)	18.9±10.0 (3.6-63.0)	63.2±1.5 (18.1-98.0)	1.7± 0.4 (0-64.0)	34.5±1.5 (0.9-80.2)	0.55±0.1 (0-5.0)
Sar (Sm) (45)	56.6±1.6 (22.5-82.5)	31.5±23.9 (6.4-167.1)	74.9±2.5 (33.0-98.8)	1.2± 0.2 (0-4.8)	23.0±1.8 (0-67.0)	0.7±0.2 (0-5.1)
EAA (Nsm) (34)	46.4±1.8 (25.0-67.5)	41.7±25.6 (3.4-110.9)	38.2±1.5 (5.2-75.1)	4.3±0.6 (0.2-12.4)	58.1±2.6 (14.6-83.2)	2.6±0.5 (0-14.3)
EAA (Sm) (4)	43.4±4.6 (31.0-52.5)	54.6±34.6 (30.0-105.7)	57.3±5.6 (41.1-72.8)	5.8±1.2 (2.6-8.0)	40.8±7.5 (19.8-53.9)	3.3±2.3 (0.5-10.3)
IPF (Nsm) (27)	42.6±2.4 (12.5-65.0)	24.2±29.5 (5.5-168.8)	67.8±3.7 (44.8-91.7)	11.7±1.9 (0.9-39.5)	13.8±3.0 (0.9-43.6)	6.4 ±1.8 (0-38.6)
IPF (Sm) (17)	46.1±4.2 (30.0-70.0)	51.9±25.2 (5.5-168.8)	63.3± 8.5 (8.1-88.8)	18.9± 8.9 (0.2-89.6)	10.2±2.7 (0.1-20.0)	7.6±2.8 (0.3-48.2)
Infectious (Nsm+Sm) (31)	28.9±5.1 (7.5-67.5)	329.1±84.8 (7-2432)	7.0±1.3 (0-25.0)	90.5±1.5 (68.8-99.8)	1.9±0.5 (0-10.8)	0.04±0.3 (0-0.8)

Data are expressed as mean ± standard error of the mean (SEM) and range in parentheses. Definition of abbreviations: n=number of cases; TCC=total cell count; AMs=alveolar macrophages; PMNs= polymorphonuclear neutrophils; Lym=lymphocytes; Eos=eosinophils; Sar=sarcoidosis; EAA=extrinsic allergic alveolitis; IPF=idiopathic pulmonary fibrosis; NSm=nonsmokers; Sm=smokers. *percentages of the total cell count

the explanatory variables derived from BALF used in this study for distinguishing pulmonary diseases of infectious and non-infectious origin were: yield or recovery ((out/in) x 100% of the BALF), total cell count (TCC) x 10⁴.mL⁻¹, the percentages of eosinophils, polymorphonuclear neutrophils (PMNs), lymphocytes and alveolar macrophages, respectively. These variables were non-missing for 31 subjects with a pulmonary disorder of a bacterial infectious origin (Group I) and 272 subjects with non-infectious ILD (Group II). Univariate testing (using the Mann-Whitney test) showed significant differences between both groups of patients for each of these variables separately (p<0.0001). The most significant one appeared to be the percentage of PMNs (p<0.00001). No other BALF variable or demographic characteristic of the population studied was included in the discriminant analysis. If this variable was introduced in a logistic regression model, then 271 of the 272 BALF samples obtained from subjects without an infection and all 31 BALF samples obtained from patients with a bacterial pulmonary infection were predicted as such. All remaining variables were far from significant when added to the model containing the percentage of PMNs (p-values ranging from 0.42 to 0.93; likelihood ratio tests). The estimated coefficient of the percentage PMNs equals 0.1498 (log odds ratio; SE=0.0517; p<0.0001 likelihood ratio test). This means that with each percent point increase in the PMN percentage, the odds of infection is multiplied by an odds ratio of e^{0.1498} = 1.16 (odds = the probability of infection divided by 1 minus the probability of infection).

A PMN percentage exceeding the cut-off value of 65 % yields a positive predictive value of 31 true infections out of 32 predicted as such (96.9 %) and a negative predictive value of 271 true non-infections out of 271 predicted as such (100 %). The sensitivity and specificity are, respectively, 100 % (31 correctly predicted out of 31 true infections) and 99.6 % (271 correctly predicted out of 272 true non-infections).

Additionally, the constructed logistic regression equation using several BALF parameters as variables to provide the most likely diagnosis [8,9] revealed a correct classification in 91.2% of the cases in the learning set and 94.5% in a test set (population patients suffering from ILD in another hospital, n=128) used to validate the program, respectively [9].

Discussion

This study demonstrated that the cellular profile of BALF samples of bacterial infectious aetiology appeared to be significantly different from samples of non-infectious aetiology. Notably, just one variable, e.g. the percentage of PMNs, allowed to distinguish between bacterial infections and non-infectious disorders. The agreement of predicted with the actual diagnostic group membership was 99.67% for this distinction. The previous equation [8] has not changed at all, but a preliminary analysis using a single cut-off value for the percentage PMNs (65%) was performed to rule out infection (before the multivariate equation was applied to diagnose the type of ILD). The reason for having chosen this conservative and theoretically sub-optimal strategy is that not all variables used for discrimination within Group II were available in all cases of Group I (e.g., smoking). Considering Group I a fourth disease would then have invalidated the prediction rules used for discriminating the three diseases within Group II. Another reason is that with only two centres there is the risk that the discriminatory power of distinguishing Group I from Group II is confounded by a potential accuracy bias between the laboratories of both centres. It would not be right to sacrifice the earlier well-validated model in Group II for this. Additionally, 91.2% of the cases within the non-infectious group were correctly classified with respect to their actual ILD diagnosis (sarcoidosis, EAA or IPF) [8,9]. Note that not a predominant cell type present in BALF, e.g. the percentage of lymphocytes nor the CD4/CD8 ratio, but a combination of features, an elevated total cell count, predominantly lymphocytes together with a nearly normal percentage of eosinophils and PMNs and the lack of plasma cells appeared to be important to distinguish the most likely diagnosis sarcoidosis in a certain case from an infectious aetiology, EAA and IPF, respectively.

In addition to their defensive role, PMNs have been implicated more recently in injurious processes associated with both acute and chronic pulmonary diseases [10,13]. In the normal lung, PMNs are commonly absent. However, in certain conditions, PMNs can accumulate within the lung structures [10,13,14]. Until now little attention has been paid to the usefulness of this particular cell in distinguishing BALF samples of infectious aetiology from non-infectious aetiology. Previously KIRTLAND *et al.* [15] found that less than 50% PMNs in BALF had a 100% negative predictive value for pneumonia. MARQUETTE *et al.* [16] also found increased PMNs in patients with pneumonia ($87 \pm 13\%$) in comparison to patients without pneumonia ($49 \pm 32\%$). In line with this, an increased number of PMNs was found in BALF samples of infectious aetiology (Group I) compared to the group of non-infectious aetiology (Group II) [10]. The percentage of PMNs demonstrated a high specificity in this study. However, we realize that one of the important limitations of the present analysis is that it not allows to distinguish other pulmonary disorders with a neutrophil excess in BALF such as acute respiratory distress syndrome (ARDS) [17-20], acute interstitial pneumonia (AIP) [21], severe IPF, nor Sweet's syndrome with pulmonary infiltration associated with myelodysplasia [6] from active bacterial pulmonary infections. Exclusion of infectious pneumonias is of great clinical importance as these disorders require a different therapeutic approach. Together with the clinical history and other investigations, identifying numerous haemosiderin-laden macrophages may be indicative of acute lung injury diffuse alveolar damage (DAD), associated with alveolar haemorrhage syndromes [3,4,7], underlying systemic diseases (e.g. connective tissue disease and vasculitis), toxic inhalants, and drugs that may cause DAD [21]). Future studies are required to evaluate whether certain BALF features are helpful in establishing the correct diagnosis [7,22,23]. Furthermore, the cellular profile of BALF obtained from patients suffering from other infections including viral infections, *Pneumocystis carinii* and *Mycobacterium tuberculosis* should be evaluated as well.

To achieve the highest diagnostic effort, BALF analysis should include appropriate cellular analysis (easily discernible on MGG stained cytocentrifuged preparations), additional Perls' stain for haemosiderin visualisation or iron staining as well as microbiological evaluation. To date, in an appropriate clinical setting, careful BALF analysis may contribute to the diagnosis of various ILD, including alveolar proteinosis, pulmonary histiocytosis X, EAA, drug-induced pneumonitis, diffuse alveolar damage, eosinophilic pneumonia, BOOP, IPF and sarcoidosis [4,9,24,25]. Furthermore, in critical ill patients the presence of more than one cause of the pulmonary damage, such as an infection in combination with a drug-induced pneumonitis should always be considered and an extensive review of the clinical record is mandatory to allow reliable interpretation of BALF analysis results [7].

As mentioned above, one of the limitations of the presented computer program is that it only includes the three most frequent ILD, i.e. sarcoidosis, IPF and EAA, and bacterial pneumonia. One should be aware of this limitation, and, therefore always look carefully at the BALF cytology. How the diagnostic accuracy of the program would be, if other causes of ILD would also be included, such as acute lung injury, bronchiolitis obliterans with organizing pneumonia (BOOP), or eosinophilic lung diseases as well as viral infections, is unknown at present and has to be explored. To date, the cellular BALF profile of a drug-induced hypersensitivity reaction appeared to be similar to the EAA profile (data not shown). Moreover, a high number of eosinophils (more than 25%) in BALF makes an eosinophilic lung disease reasonable. The presence of Reed Sternberg cells makes a lymphoma highly likely [4,24]. A milky aspect of the BALF justified the suspicion of alveolar proteinosis [4]. Furthermore, the presence of

numerous iron-laden macrophages points to causes of disorders associated with DAD. Additional iron staining may be helpful to distinguish disorders with DAD from other ILD, e.g. pulmonary vascular disorders such as M. Wegener from sarcoidosis as both may present with a lymphocytosis in BALF [26]. Moreover, the value of BALF cytology findings for the diagnosis of non-infectious conditions in ICU patients with suspected pneumonia was demonstrated [7]. The medical history is extremely important to identify the possible cause of the pulmonary damage in those cases. In line with this, one should always interpret the BALF data in the context of other relevant information such as the occupational history of the patient, as well as other clinical parameters, such as the presence of precipitines, an elevated serum ACE level, HRCT features etc. Therefore, we want to emphasize the importance of careful assessment of the all cytological BALF characteristics in order to improve the diagnostic accuracy in patients with diffuse lung damage. The computer model should be used with caution considering the limited number of diseases included in the model. However, the included ILD *i.e.* sarcoidosis, IPF and EAA cover for about 80-90 percent of all ILD.

This study only focussed on BALF data obtained in the diagnostic work-up of patients suffering from diffuse lung diseases. Although the accuracy of high resolution computed tomography (HRCT) in the differential diagnosis of these patients has been widely appreciated [1,2,27-29], no HRCT data were included in this model. It would be very helpful if a HRCT score could be used to classify a certain disorder. However, the attempts made to evaluate the usefulness of HRCT in characterize disease were mainly descriptive [27]. Moreover, the appearances on HRCT were correlated with disease activity in fibrosing alveolitis [29-31], acute farmer's lung [32], sarcoidosis [33, 34] and Wegener's granulomatosis [26]. It would be of great interest to develop a valuable diagnostic quantitative HRCT score which discriminates between certain ILD. Additionally, a prospective study should address the effect of including this score in a logistic regression model upon the diagnostic probabilities of the BALF data presented in this study. Including more clinical data in the model may further improve the diagnostic accuracy of patients suffering from diffuse lung diseases.

In conclusion, when applied according to standardized protocols, and considered in the context of other information from conventional ancillary diagnostic tests, such as a HRCT scan, BAL appears to be useful in the diagnosis of diffuse lung diseases. The updated computer program provides a reliable prediction of the diagnosis of a bacterial pneumonia or one of the three most frequent interstitial lung diseases, and is thought to improve the diagnostic power of BALF analysis in conjunction with other important diagnostic procedures.

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