CHAPTER 8

Summary and general discussion

Pulmonary diseases have traditionally been evaluated by laboratory tests, lung function tests, imaging procedures and tissue biopsie.[1] Bronchoalveolar lavage (BAL) represents an additional tool in assessing the health status of the lung. BAL is a procedure in wich the bronchoalveolar region of the respiratory tract is lavaged with an isotonic salt solution, It samples cells and solutes from the epithelial layer of the lower respiratory tract. After its introduction as a research tool, BAL has been appreciated extensively for clinical applications in the field of infections and intestitial lung diseases. Diagnostic application of BAL fluid analysis is mainly based on cellular characteristics of the fluid, therefore, appropriate cellular analysis is mandatory. Normally, BAL fluid samples from healthy non-smoking controls contain alveolar macrophages, lymphocytes, and, to a lesser extent, polymorphonuclear neutrophils (PMNs), eosinophils and mast cells.

Although the value of BAL fluid cytology in the assessment of interstitial lung disease and pulmonary infections is apparent, it should be realised that the actual determination of the cell types does require technical skill and training, and that the entire BAL fluid processing requires considerable time and effort.

The procedure of cytocentrifugation has been used for many years in haematology and cytology laboratories, as it enables excellent observation of cell morphology in specimens of low volume. During the past decades, applications of cytocentrifugation have been developed for diagnostic microbiology. Among others, these applications included screening for urinary tract infections, visualisation of bacteria in normally sterile body fluids, and detection of acid-fast bacilli in sputum preparations.[2-4] Despite encouraging results, cytocentrifugation has failed to become a widespread technique in diagnostic microbiology.

The diagnostic value of cytology largely depends on the possibilities for standardising the procedures of cytocentrifugation. Indeed, technical factors interfering with the cytocentrifugation process may cause distortion of the BAL fluid differential cell count. Such errors include the “bull’s eye” appearance of the cytocentrifuge spot (due to low sample volumes in the cytospin chamber), and the “cresent shaped” apprearance of this spot (due to a delayed start of the centriguge after loading of the chambers).[5]

Although process has been made in non-morphologic diagnosis (e.g. flow cytometry and polymerase chain reaction-techniques), the microscopic identification of cells and pulmonary pathogens constitutes the cornerstone of BAL fluid analysis in the daily practice of the diagnostic laboratory, and it is likely to remain so in the near future. Indeed, analysis of the cellular profile in BAL fluid samples can give more insight into the underlying lung disease. The of refined techniques of specimen processing and microscopy not only offers fast and reliable diagnosis, but also fits the needs for cost-accountable specimen management and round-the clock patient care.

Despite the recommendations of the European Society of Pneumonology and those of the American Thoracic Society,[6,7] further study on standardisation oof the cytocentrifugation process is necessary.

Chapter 2[8] and 3[9] were conceived as a spin-off of this search for standardisation. In these chapters, the influence of the cytocentrifugation parameters on the differential cell count of BAL fluid samples was investigated. In **chapter** 2[8], the impact of three cytocentrifugation parameters (i.e. speed, time and acceleration rate) on the BAL fluid differential cell count was investigated. As cytocentrifugation entails selective loss of lymphocytes, the recovery rate of these cells at various cytocentrifugation conditions was of special interest.[10,11] A cytocentrifugation speed of 1200 revolutions per minute (rpm), and a duration of 10 minuytes ascertained the highest recovery rate of lymphocytes. At intermediate (1200 rpm) and high speed (2000 rpm) conditions however, morphological cell damage became apparent, and thus we elected to use the low cytocentrifugation speed as the one preferred for BAL fluid cytology. In **chapter** 3[9], it was concluded that lymphocytes and alveolar macrophages were not randomly distributed on the cytocentrifuge spot, and that the centre of the cytocentrifuge spot appeared to be the most reliable area for perfomance of the differential cell count. Although the findings in both chapters were statistically significant, their clinical significance remains debatable. The mean absolute differences in lymphocyte percentages for the different quadrants (**chapter** 3)[9] and for the different cytocentrifugation conditions (**chapter** 2)[8] were small, but there were many individual BAL fluid samples for which the absolute difference exceeded 10%. Although no tolerance limits have been defined for interassay variability of BAL fluid differenbtial cell counts, a 10% difference is considered technically sifnificant when assessing interlaboratory variabilities.[12] Further investigation on the limits of interassay variability of BAL fluid differential cell count, without clinical impact, should be done and will increase the application of BAL fluid cytology in the daily practice.

Chapters 4[13] and 5[14] present refinements of the previously published recommen-dations on the number of BAL fluid cells to be differentiated. In

chapter 4[13], it was demonstrated that a 200-cell count (which the American Thoracic Society proposed as the minimun number of cells to be enumerated)[6], only warranted a reliable count of PMNs and alveolar macrophages. When increasing the number of cells counted to a total of 300, the lymphocytes and eosinophils were also reliably enumerated. However, the percentages of mast cells, plasma cells, squamous and bronchial epithelial cells were not reliable estimated, even at a count of 500 cells by one observer. On May-Grünwald-Giemsa stained preparations, it appeared that reliable counting of mast cells was not possible, and therefore caution should be excercised when interpreting mast cell percentages. Extended microscopic screening of BAL fluid cytocentrifuged preparations was recommended, in order to evaluate the presence of epithelial cells and plasma cells.

Chapter 5[14] demonstrated that MGG stained preparations offered a more reliable counting of infected cells than did the Gram and the Acridine Orange stained preparations. Reliable estimination of the percentage of infected cells was achieved at a count of 200 cells. The results obtained in chapters 4 and 5 may be regarded as a further attempt to standardise the BAL fluid differential cell count. The studies presented in **chapter** 4[13] and 5[14] should be complemented by a detailed investigation aof omter-laboratory differences in the processing of BAL fluid samples, in order to achieve a better standardisation of the BAL differential cell counts.[15]

From the scope of instrumentation and automation, several attempts have been made to simplify the processing of BAL fluid samples in the diagnostic setting. Calibration of a Coulter Counter D apparatus enabled fast and accurate electronic measurement of the total cell count of BAL fluid samples, and a commercial Dip Slide method compared favourably to conventional quantitative culture methods.[16,17] In line with these evolutions, a commercial Leukocyte esterase strip, designed for urinalysis, was evaluated for its ability to detect elevated numbers of PMNs in BAL fluid samples. The results of this study, presented in **chapter 6**[18], showed that the Multistix 7 reagent strip proved to be useful as a rapid screening test for the BAL luid PMNs percentage. However from the viewpoint of diagnostic and analytical sensitivity, further study on the false-negative readings and refinements of the reading scale are needed. Furthermore, adaptations of the other Multistix parameters to the BAL fluid ranges are desired. Although BAL fluid samples on their own do not represent a large field of application (and sale) for such reagent strip, they would be a useful adjunct to conventional microscopy in those situations where trained cytologists are not available.

In **chapter 7**[19], the usefulness of BAL fluid cytology was assessed in intensive care patients suspected of having pneumonia. In these patients, pulmonary densities noted on chest radiograph are numerous, and non-infectious

disorders may be just as prevalent at those produces by organisms[20] From the study presented in **chapter 7**[19], it was clear that BAL fluid cytology may offer valuable information in the detection of at least part of these non-infectious conditions, Readily discernible cytological findings such as haemosiderin loaden macrophages, eosinophils and malignant cells clearly pointed to a non-infectious condition such as aspiration of gastric contents, the Adult Respiratory Distress Syndrome, diffuse alveolar haemorrhage and lymphangitis carcinomatosa. It is important, however, to realise that none of these findings was exclusive, a reason why thorough microbiological examination of the BAL fluid samples is still required in order to rule out an infectious origin.

In summary

1. Optimal recovery of the different BAL fluid cell types combined with excellent cytomorphology can be achieved by cytocentrifugation at internediate speed (650 rpm), for a duration of 10 minutes and at the low acceleration rate.
2. For reliable estimation of the BAL f;uid lymphocyte and alveolar macrophage numbers, the differential cell count of cytocentrifuged BAL fluid samples should be performed in a circular pattern around the center of cytocentrifuge spot.
3. Reliable estimation of the number of PMNs, alveolar macrophages, lymphocytes and eosinophils in cytocentrifuged BAL fluid samples is reached at a count of 300 cells by one observer. Extended microscopic screening is recommended to evaluate the presence of epithelial cells (low magnification) and plasma cells (high magnification).
4. For unumeration of the infected cells in cytocentrifuged BAL fluid samples, the MGG stain is the preferred stain, and reliable enumeration by a single observer is achieved at a count of 200 cells.
5. For estamation of the BAL fluid PMN count, the leukocyte esterase reagent (LE) Multistix 7 reagent strip (designed for urinanalysis) can be of adjunct value: negative LE rading consistently predict a BAL fluid PMN count of <20%, and the "+++" LE category predict PMN counts above and below 50% with a sensitivity of 71% and a specificity of 97%.
6. In intensive care patients suspected of having pneumonia, BAL fluid cytological findings such as >20% haemosiderin loaden macrophages, the presence of activated lymphocytes, plasma cells, >5% eosinophils, a preponderance of foamy macrophages, reactive type II pneumocytes and malignant cells, point to a non-infectious origin of the symptoms.

Further refinements of these findings in a prospective study will increase the diagnostic accuracy of the BAL fluid cytology in the prediction of non-infectious as well as infectious conditions. The studies in the previous chapters illustrated the value of a multidisciplinary approach in the processing and interpretation of BAL fluid cytology. Excellent technical and statistical support enabled to refine BAL fluid processing. However, appropriate clinical information is mandatory to improve the diagnostic accuracy of BAL fluid analysis. When applied according to standardized protocols, and considered in the context of other diagnostic tests and appropriate clinical information, BAL appears to be useful in the diagnosis of certain lung diseases. Attempts have to be made to further improve the procedure and explore the value of the procedure for clinical as well as research application.

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