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PALESTRA/LECTURE

Diferenciais celulares e subpopulações linfocitárias no LBA da pneumonia de hipersensibilidade

Bronchoalveolar Lavage Cell Differentials and Lymphocyte Subsets in Hypersensitivity Pneumonitis

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RESUMO

Faz-se uma profunda revisão das características do Lavado Broncoalveolar nas Pneumonias de Hipersensibilidade (PH). Discute-se o perfil do LBA nas diferentes fases de PH, da relação T4/T8 e dos marcadores de actividades dos linfocitos. Seguidamente são apresentados os resultados do LBA em indivíduos expostos assintomáticos e os padrões de alveolite que aparecem no *follow-up* destes doentes. Finalmente defende-se a relevância diagnóstica de LBA na Pneumonia de Hipersensibilidade.

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Palavras-chave: LBA, subpopulações linfocitárias, pneumonia de hipersensiblidade

ABSTRACT

A thorough review on BAL data in hipersensitivity pneumonitis (HP) is made. The BAL cell profile on the different phases of HP is discussed, the CD4/CD8 ratio, the lung Tcell markers of activation. Then the BAL findings in asymptomatic exposed individual are addressed as the pattern of alveolitis during follow-up. Finally the diagnostic relevance of the BAL findings in HP is defended.

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Key-words: BAL, Limphocyte subsets, Hypersensitivity pneumonitis

Bronchoalveolar lavage (BAL) is now widely used in the diagnostic evaluation of interstitial lung disease. Initially, the technique was applied for research purpose only to gain information about the immunopathogenesis of lung diseases. In recent years, the diagnostic potential of BAL has been increasingly appreciated. The quantity and composition of cells collected by BAL are modified in disease states. Numerous studies have investigated the cellular findings in the BAL fluid of patients with HP.

Total cell count and cell differentials

The classical changes in the cellular composition of BAL in HP can first be seen in the subacute or chronic phase of the disease, more than one week following the acute exposure. The total cell count is markedly increased, approximately five to seven fold that observed in controls (Table 1). This is mainly caused by a large increase in the lymphocyte count, usually to more than 50% of total cells. The reported mean values for lymphocytes from the literature range between 50 and 70 % (Table 2). The increase of lymphocytes represents the most striking finding during the entire disease process (1-9).

In the very early acute phase of the disease, soon after the exposure to specific antigen or after the challenge with HP causing antigens, the neutrophils are markedly increased (10,11). Follow-up data showed that this increase in neutrophils (within the first 24 to 48 hours after challenge) returns to normal or only mildly increased values within the following 8 days after challenge. In addition, eosinophils and mast cells may be mildly elevated (5,7,8,9).

A more specific finding is the increase in plasma cells (12,13). This cell type was found in a low percentage in 18 of 30 patients with bird keeper's disease,

TABLE 1

Total cell count in BAL from published studies on hypersensitivity pneumonitis (HP)

	HP	Exposed	Controls
	symptom.	asympt.	
Leatherman (2) (300 ml) ($\times 10^6$)	71±20	36±11	10±1
Costabel (3) (100ml) ($\times 10^6$)	42±10		6±2
Semenzato (4) (100 to 150 ml) ($\times 10^6$)	62±16	20±7	12±1
Soler (5) ($\times 10^3$/ml)	990±485		158 ±65
Laviolette (8) ($\times 10^3$/ml)	558±256	130 ±88	65 ±16
Pesci (7) ($\times 10^3$/ml)	542±211		175 ±37
Johnson (6) ($\times 10^3$/ml)	575	502	

Table 2

Lymphocyte percentage in BAL from published studies on hypersensitivity pneumonitis (HP)

		HP	Exposed	Controls
		symptom.	asympt	
Leatherman (2)	%	65±4	48 ±5	16 ±2
	CD4/CD8	0.8±0.2	0.7 ±0.2	1.8 ±0.2
Costabel (3)	%	69±15		8 ±3
	CD4/CD8	1.0±0.7		1.8 ±0.7
Semenzato (4)	%	78±5	36 ±6	8 ±1
	CD4/CD8	0.5±0.1	0.9 ±0.2	2.0 ±0.2
Soler (5)	%	66±18		11 ±5
	CD4/CD8	0.9±0.7		1.8 ±0.6
Laviolette (8)	%	59±15	19 ±15	6 ±4
Pesci (7)	%	56 ±18		7 ±1
Johnson (6)	%	44 ±14	29 ±19	
		0.9 ±0.3	1.1 ±0.6	

values ranging from 0.1 to 3.9 % in this study (13). Other morphologic findings include signs of activation of T-cells (folded nuclei) and foamy macrophages (14).

Lymphocyte subsets

In absolute numbers, both CD4 and CD8 positive T-lymphocytes are drastically increased compared to healthy subjects. Only a few BAL lymphocytes express B-cell related surface markers. Regarding percentages of T-cell subsets, however, in the majority of cases there is a predominant expansion of CD8+ T-cells bearing the cytotoxic/suppressor phenotype, resulting in a low CD4/CD8 ratio. The different series reported in the literature show no consistent behavior, however. Most studies show a significantly decrease in the CD4/CD8 ratio, with mean values ranging between 0.5 and 1.0 (6 different groups of researchers) (2,4,5,6,14,15). Two studies found that CD4/CD8 ratios were borderline (1.3 and 1.5, respectively) (9,16). In Japan, a normal ratio of 2.0 has been reported for ventilation HP, and even an increased mean ratio of 4.4 for farmer's lung (15). The reasons for this discrepancy in reported CD4/CD8 ratios are unclear. Several explanations are possible and include first different disease manifestations (acute versus chronic form), second the timing of BAL investigations in relation to the last antigen exposure, and third the type of antigen causing the disease.

When analyzing the major literature reports, CD4/CD8 ratios are higher in the acute (13,16,17) versus the chronic form (2,14,17), are higher very shortly after the last antigen exposure (within 24 hours) and lowest between 7 and 30 days since last exposure (5, 9) (Table 3). The type of antigen (farmers' antigen, birds, etc.) does not seem to play a role. Although mean values of the CD4/CD8 ratio in most studies are low, in an individual patient the ratio may be within the normal range or even increased. Own data show that in 18 of 30 patients with HP the ratio

Table 3

CD4/CD8 ratios (mean values) in hypersensitivity pneumonitis in relation to acute or chronic disease and in relation to time elapsed since last antigen exposure

Condition	CD4/CD8 ratio		
acute	1.3 (ref. 16)	1.5 (ref. 9)	1.8 (ref. 17)
chronic	0.8 (ref. .2)	0.9 (ref. 17)	1.0 (ref. 14)
last antigen 1-2 days 10-30days	Soler (5) 1.3 0.4	Drent (9) 1.8 1.0	

is decreased (below 1.0), in 11 of 30 in the normal range (1.0 to 3.5) and only in 1 patient elevated (18). Similarly, Drent et al showed that in 21 of 45 patients the ratio is below 1.0, in 25 of 45 patients in the above mentioned normal range, and in 5 of 45 patients increased above 3.5 (9).

Lung T-cells in HP apparently are highly activated, as evidenced by their phenotypic characteristics. They show increased expression (both in percentage and absolute number) of HLA-DR related antigens. An increase of very late activation antigen (VLA1+) and γ/δ-T-cell receptor positive cells has also been found (19). The number and percentage of cells bearing proliferation associated markers (CD71 and CD25) is quite low (4,14,18).

Natural killer (NK) related markers were analyzed in detail by Semenzato et al. They found that CD56 and CD57 positive cells are significantly increased compared with controls. The increased expression of CD57 (172) was confirmed by another group. The CD16 NK-related phenotype is not expressed on lung T-cells in HP (19).

Asymptomatic exposed individuals

In these individuals, although regularly exposed to HP antigens, neither clinical features nor radiological or functional abnormalities are present, even when exposed on the farm or as pigeon breeders. Frequently, however, serum precipitin are positive, and BAL shows signs of a lymphocytic alveolitis (2,4,6, 16,20). This subclinical alveolitis is more frequent in farmers with positive serum precipitins than in precipitin negative subjects (20). Usually, the mean value of the BAL lymphocyte percentage is lower than in symptomatic disease (2,4,6,16,20). The CD4/CD8 ratio tends to be somewhat higher than in patients with manifest disease (4,6,16).

A follow-up study of subclinical alveolitis in asymptomatic farmers for two years showed that the BAL lymphocytosis persisted and that no subject developed farmer's lung disease (21). Therefore a pathological BAL finding with increased percentages of lymphocytes in exposed but otherwise healthy persons has to be considered as evidence of sensitization, but not of disease, as is true for serum precipitins.

The pattern of alveolitis during the follow-up

Trentin et al. showed that the BAL lymphocytosis persisted for up to 30 months in farmer's lung, both in patients who continued to be exposed to specific antigens at work as well as in patients who were not further exposed but continued to live in agricultural environments (22). Interestingly, the CD4/CD8 ratio remained low in those patients with persistent exposure at work, whereas in the other group the CD4/CD8 ratio returned to normal after 6 months (22). Similarly, Costabel and co-workers showed that the removal from antigenic exposure decreased the intensity of alveolitis, as reflected by a lower BAL lymphocytosis in patients not exposed to HP antigens anymore. The reduction in the BAL lymphocytes took a long time, and only after three years the mean lymphocyte percentage was close to the normal range (18,23). Johnston and co-workers showed a more rapid return to normal after removal from antigen in pigeon breeders lung. After only three weeks they observed

a fall of the lymphocyte percentage below 20 % in 5 of 6 patients studied (6). Drent and co-workers performed a cross sectional analysis and related the BAL findings to the time elapsed since last antigen exposure (9). They also found a lower lymphocyte percentage in those individuals having avoided antigen between 1 to 12 months (41% lymphocytes) versus those having avoided antigen only for 2 to 7 days (61% lymphocytes). A similar reduction was seen for the number and percentage of eosinophils, mast cells and plasma cells in this study. The data reported by different groups are not necessarily contradictory as they may be related to different study populations. In addition, it is often difficult to precisely distinguish between patients who managed to avoid antigen exposure completely and those who may have residual exposed to minor amounts of antigens when still living in the agricultural environment or visiting houses where birds are kept in the case of bird keeper's lung.

Diagnostic relevance of BAL findings in HP

The increase in the absolute and relative number of lymphocytes is the most consistent BAL finding in HP. Usually, the lymphocyte percentage is higher than 40% (Fig. 1). In fact, patients with symptomatic disease and nórmal findings or isolated increase in neutrophils or eosinophils have not been reported. From this we conclude thaf a lack of BAL lymphocytosis virtually excludes the diagnosis of HP. A low lymphocytosis in the range between 15 and 30% makes the diagnosis unlikely.

A lymphocytic alveolitis, associated with an increase in CD8 positive cells, is a nonspecific finding, however, which is also observed in patients with pneumonia associated with collagen vascular diseases, bronchiolitis obliterans organizing pneumonia (BOOP), drug induced pneumonitis and patients with HIV-infection, amongst other conditions (24). The presence of plasma cells in BAL has also been observed in drug induced pneumonitis, BOOP, chronic

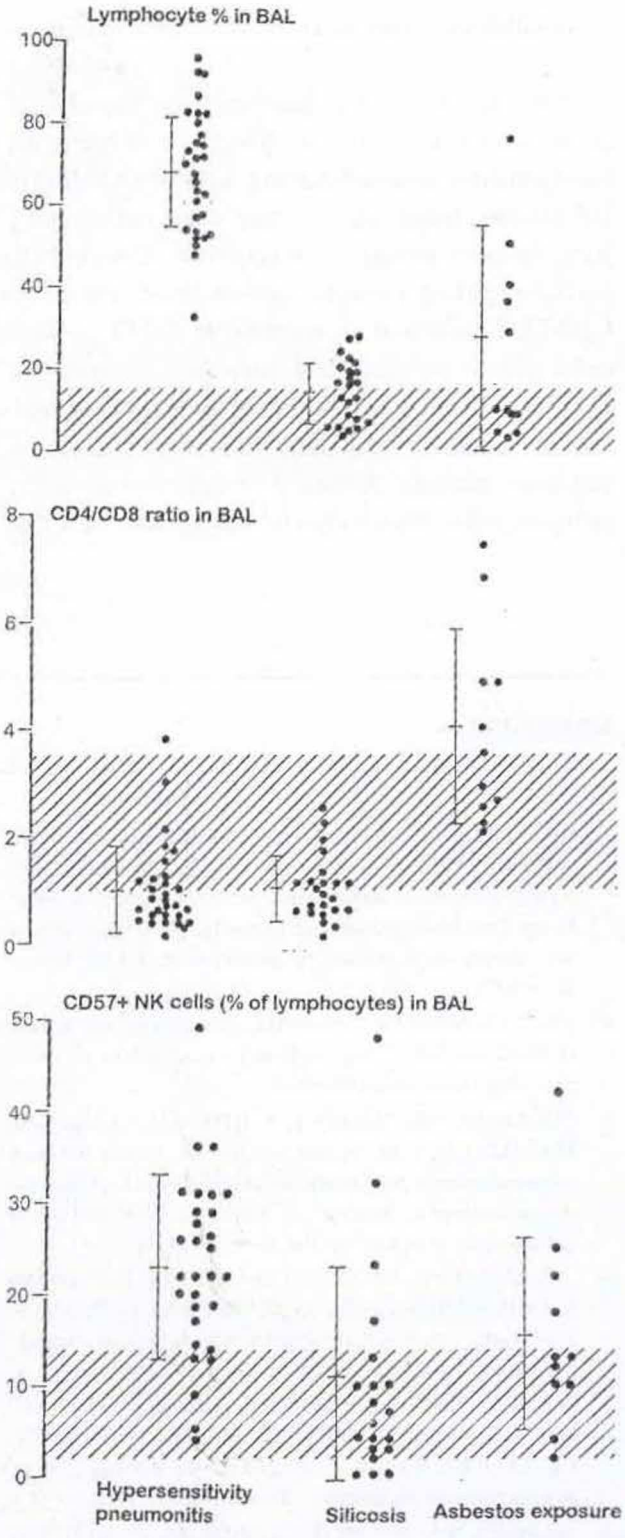

Fig.1 – BAL findings in hypersensitivity pneumonitis in comparison with those in inorganic dust disease (from reference 18 with permission)

eosinophilic pneumonia, and hematologic malignancies.

Although not being specific, from the clinical point of view BAL has the advantage of being the most sensitive tool in detecting signs of alveolitis in HP patients, more sensitive than chest radiography, lung function testing or precipitins. A very high percentage of lymphocytes in association with a low CD4/CD8 ratio and an increase in CD57 positive cells, with plasma cells and mast cells, is suggestive of HP and should make further investigations obligatory in a patient with interstitial lung disease of unknown etiology. A careful re-examination of the occupational or environmental history and the screening of serum precipitins might then reveal previously unknown sources of antigen exposure.

In summary, the following conclusions about the clinical significance of BAL findings in HP are relevant:

1. A lack of a BAL lymphocytosis excludes HP.
2. BAL is the most sensitive tool in detecting signs of alveolitis.
3. BAL is not able to differentiate between symptomatic interstitial lung disease and subclinical alveolitis in a given patient.
4. In the follow-up, persistent BAL abnormalities indicate that complete allergen avoidance has not been achieved.

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