BRONCHOALVEOLAR LAVAGE AND SILICOSIS PATHOGENESIS

A. TELES DE ARAÚJO • Ana Cristina Mendes • Jorge Monteiro • Gina Duarte • M. Freitas e Costa

Clínica de Doencas Pulmonares (F.M.L.) and Centro de Investigacão CnL3 (INIC), Lisboa, Portugal

INTRODUCTION

Inhalation of silica can lead to chronic inflammatory interstitial lung injury susceptible to progress to silicosis with clinical expression.

The modern theories about pathogenesis of silicosis are clearly synthesized by Davis[7] and consider that the silica-laden alveolar macrophages maintain viability and phagocytotic ability and once activated by the noxious dust produce pro-inflammatory mediators which are able to initiate the disease pathogenic pathways, such as interleukin, chemotaxis, macrophage derived growth factor.

The alveolar macrophage of these patients is also able to release or provoke the releasing of Paf-acether[15] and histamine mediators that also interfere in the disease process.

As the silica is indestructible and the lung tissue clearance of silica dust is very slow[4] the chronically activated silica-laden macrophages perpetuate the inflammatory process, continuously releasing the above mentioned mediators.

In these patients, besides the inflammatory process, there are immumologic mechanisms locally expressed by an increasing in the T-lymphocytes.[3,7,17]

All these facts suggests the existence in the chronic silica exposed workers of an alveolitis what had been confirmed by different authors[14,12,7,13] and evoke the interest of the bronchoalveolar lavage in their study.

In this article we studied a group of 34 long-term silica-exposed workers with a broad spectrum of disease manifestations through clinical chest X-ray, Functional and Bronchoalveolar lavage fluid investigations in order to achieve a better comprehension of the disease pathogenesis and evaluate the eventual correlation between the BAL data and clinical manifestations.

MATERIALS AND METHODS

Patients

Thirty-four patients were studied being 27 males and 7 females with ages ranging between 31 and 78 years old ($\bar{X} = 48 \pm 13$). Fifteen of the patients were smokers and none of them had previous history of any other pulmonary disease. All were current workers in industry with exposition at least in the week prior to evaluation.

As generally accepted[7] the diagnosis of silicosis was based on a history of a prolonged exposure to silica dust and a chest X-ray showing shadows compatibles with silicosis in category 1 or above according to ILO classification.[8]

All patients were submitted to a standard posterior anterior chest X-ray, read by 3 observers according to ILO classification and to a functional study by global body plethysmography.

The patients were divided in two groups. The group I,was composed of 22 patients without or only with bronchial complaints, radiograph of the category 1 and normal functional tests or with a mild obstructive syndrome. The group II was constituted by 12 patients with complaints suggesting interstitial involvement, chest X-ray above category 2 and frequently with coalescence of lesions and functionally with volumetric restriction.

Controls

As controls we used 6 normal volunteer without exposition to silica containing dust, with a distribution by sex and ages similar to patients. Two of the controls were smokers.

Bronchoalveolar Lavage (BAL)

The BAL procedures and analyses have been previously described.[11] Briefly bronchoalveolar lavage was performed by slow infusion of 4 × 50 ml 37°C aliquots of saline solution through a 50 ml Luer-lock Syringe attached to the bronchofiberscope, followed by gentle syringe aspiration of the effluent. The BAL was performed in one of the middle lobe sub-segments.

After remotion of mucus cells were counted in a hemocytometer and cytocentrifuge smears were prepared and stained by May-Grunwald Giemsa for identification of the cellular population.

The cellular pellet was obtained by centrifugation, 500 G at 40°C during 20 minutes, washed twice with PBS solution and resuspended in PBS solution at the final concentration of 5×10^6 cells/ml. Then the T-Lymphocytes and its subpopulation were characterized by indirect immunofluorescence after banding to specific monoclonal antibodies.

The BALF supernatants were concentrated 25 folds by ultrafiltration and the IgA and IgG dosed by radial immunodifusion (Mancinni technique).

In order to evaluate the eventual modification of the surfactant in patients and the release of active lipidic molecules, the lipidic composition of the supernatants of 8 of the patients and 4 controls were studied by thin layer chromatography and phosphorous analysis[9] and the etherlipids and Lyso-Paf-acether were assayed by washed rabbit platelet aggregability, method of Benveniste.[2]

RESULTS

The chest radiographs were classified in category pl in 65% of the patients and in above categories in 35% of the cases. Fifteen per cent of the patients presented coalescence of opacities and 30% stated hilar involvement (Table I).

The ventilatory tests were normal in 41% of the patients; 32% had obstructive syndromes, 19% restrictive syndromes and 14% combined ventilatory syndromes (Table II).

Under the defined criteria 65% of the patients must be included in category I and 35% in category II.

From the analysis of the cellularity of patients BAL effluents appears the increasing number of cells per ml, statistically significant in category II patients (Table III).

In this category of patients is almost constant the existence of an alveolitis depending on an increasing of the macrophages and Lymphocytes, mainly of these last ones which are 9 folds above the normal values. It appears also that even in category I there is a significant increase of the number of lymphocytes. Finally the number of cells, macrophages and lymphocytes is significantly higher in category II patients than in category I.

The analysis of the lymphocytic populations through monoclonal antibodies shows in category II a significant increase of the T lymphocytes, T helper lymphocytes and much more striking of the T suppressors. However percentually there is a decrease of the T helper and increase of the T suppressors leading to an inversion of T_h/T_s ratio (Table IV). The increasing of T suppressors and the inversion of T_h/T_s ratio is also evident in category I patients.

The IgA is slightly and the IgG significantly higher among patients of category II than of category I (Table V).

The analysis of the lipidic composition of surfactant makes evident a significant diminishing of the phosphatidylcholine among patients, correlating inversely with the numbers of macrophages and lymphocytes (Tables VI and VII).

In none of the studied BAL effluents Paf-acether was found. However in the patients there was a significant increase of its percursor the Lyso-Paf-acether as compared to normal controls and an exponential correlation with the number of lymphocytes was found in patients (Table VIII). No correlation between Lyso-Paf-acether and phosphatidylcholine rates was found.

DISCUSSION

From the results of BAL studies of workers exposed to silica we had studied, appear that the composition of lower respiratory tract fluid clearly reflect the clinical spectrum of the respiratory disease caused by chronic inhalation of silica containing dust.

Table I
Chest Radiographs

p = 1	65%
p > 1	35%
CONFLUENCE	15%
hi	30%

Really it had been in BAL effluents of category II patients that we had found the most impressive increasings of immunological and inflammatory effectors cells and it had been in the patients with alveolitis that the ventilatory impairment is more frequent and important.

These data are in agreement with those referenced in literature reporting either to cases with history of exposition but without disease, either to complicated forms of silicosis, being possible, in both cases, to disclose the existence of an alveolitis.[3,5,6]

Once more the central role of Alveolar Macrophage in the chronic inflammatory lung process remains evident through its potent immuno-modulatory action, its capacity of secret fibro regulatory compounds[3,10,12] and Paf-acether, once activated by occupationally relevant stimulus.[1,15,18] Indeed in category II patients there is a significant increase of the A.M. number and in both categories signs of cellular activation: increased number of A.M. with two or more nuclei and of foamy cells, spontaneous formation of rosettes A.M.—Lymphocytes and a percentage of giant cells above 2%.

This evidence of activation becomes more important once accepted the hypothesis that the silica stimulated A.M. releases in vivo Il_1. Then the T-lymphocytes activated by Il_1 enhances the secretion of macrophage activating factor, migration inhibition factor and other molecules leading to the recruitment of peripheral blood monocytes and to the proliferation of resident macrophages, amplifying and perpetuating the local inflammatory response, even after ceasing of the silica containing dust exposition.[12] This hypothesis is also corroborated by the increased number of OKM_1 positive cells we had found in BAL fluids of patients with occupational respiratory diseases caused by mineral containing dust inhalation.[6]

Another important fact to be stressed is the evidence of local cellular and humoral immune abnormalities in silicotic patients.

It is reasonable to think that the T lymphocyte stimulated by the activated A.M. releases Il_1 leading to a T-helper local proliferation. Simultaneously the A.M. also perhaps secret molecules with inhibitory functions causing an increasing in the number of T suppressor cells[12] with the objective of

Table II
Ventilatory Function

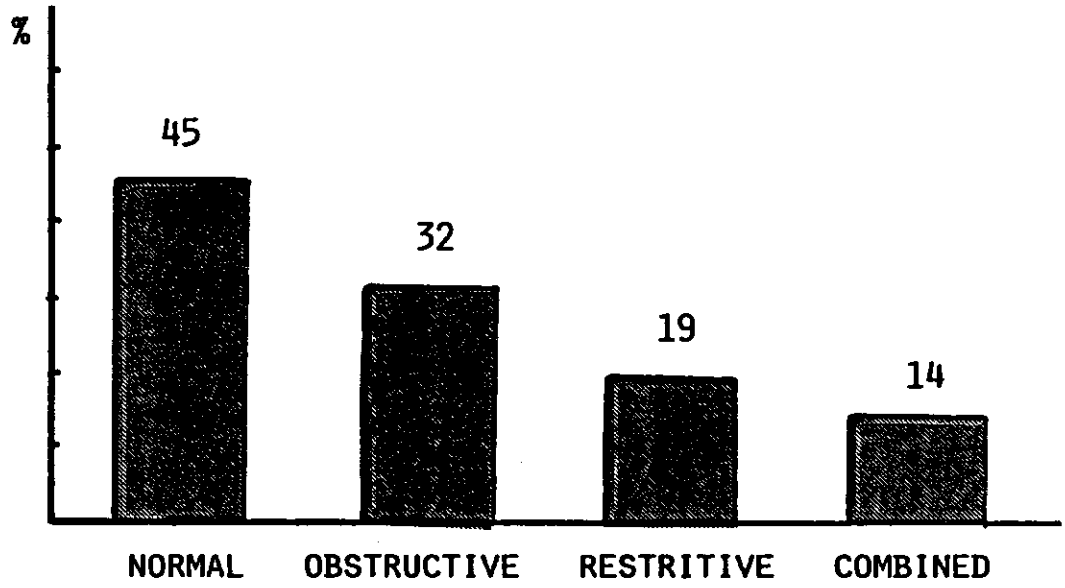


Table III
Number of Cells × 10^4/ML

	TOTAL CELLS	MACROPHAGES	LYMPHOCYTES	P.M.N.
CATEGORY I n = 6	16,7± 7,7	13,4± 6,9	3,7±2,2	0,3±0,6
CATEGORY II n = 6	44,7±14,5	28,6±13,4	15,1±5,3	1,1±2,6
CONTROLS n = 6	17,4± 4,1	15,8± 3,8	1,6±0,6	0,2±0,1

CONTR.-CAT. I	N.S.	N.S.	p < 0,02	
CONTR.-CAT.II	p < 0,001	p < 0,05	p < 0,001	N.S.
CAT.I - CAT.II	p < 0,001	p < 0,001	p < 0,001	

T - STATISTIC ANALYSIS

Table IV
T-Lymphocytes (Cells × 10^3/ML

	T_3	T_4	T_8	T_4/T_8
CATEGORY I n = 6	14,5±10,2	10,2± 5,7	16,1± 7,6	0,8±0,4
CATEGORY II n = 6	106,1±34,7	37,7±14,1	64,4±26,6	0,6±0,2
CONTROLS n = 6	10,9± 3,4	6,6± 1,9	4,1± 1,3	1,6±0,1

CONT.-CAT. I	N.S.	N.S.	$p < 0,01$	$p < 0,001$
CONT.-CAT.II	$p < 0,001$	$p < 0,001$	$p < 0,001$	$p < 0,001$
CAT.I-CAT.II	$p < 0,001$	$p < 0,01$	$p < 0,01$	N.S.

T - STATISTIC ANALYSIS

braking the immune local reaction. In mild forms of disease this equilibrium seems to be sufficient.

In complicated silicosis there are simultaneously increased number of T helper and T suppressor cells and it is possible to speculate that these immunoabnormalities contribute to the pathogenic mechanisms.

The modifications in the composition of surfactant phospholipids suggest that silica, through its cytotoxic action, damage the type II Pneumocyte with perturbation of normal surfactant functions. Once that it seems[20] that the surfactant improves the mucus transport in airways, it is possible that this is one of the responsible mechanisms for the high frequence of bronchial complaints among silicotic patients. This hypothesis is reinforced by the data of the Louisiana study on workers with complicated silicosis in which increased number of type II Pneumocyte in lavage were found.[14]

Although Paf-acether wasn't found in lung lavages of patients our data provides some evidence of an activation of the Paf-acether pathway based in the great increase in Lyso-Paf-acether rates (percursor of Paf-acether) and its correla-

Table V
Immunoglobulins

	IgG	IgA
CATEGORY I n = 6	2,3±2,6	2,1±2,8
CATEGORY II n = 6	7,5±4,3	2,0±0,7
CONTROLS n = 6	1,1±1,1	0,7±0,8

CONTR.-CAT. I	N.S.	N.S.
CONTR.-CAT.II	p < 0,001	p < 0,02
CAT.I -CAT.II	p < 0,05	N.S.

T - STATISTIC ANALYSIS

Table VI
Surfactant Phospholipids

	PHOSPHOLIPIDS TOTAL µg/ml	PHOSPHATIDILCHOLINE (%)
CONTROLS	25.4±7,8	28.2±5.9
		S**
SILICOSIS	21.6±4.0	16.5±3.6

S** p < 0,02

Table VII
Surfactant Analysis

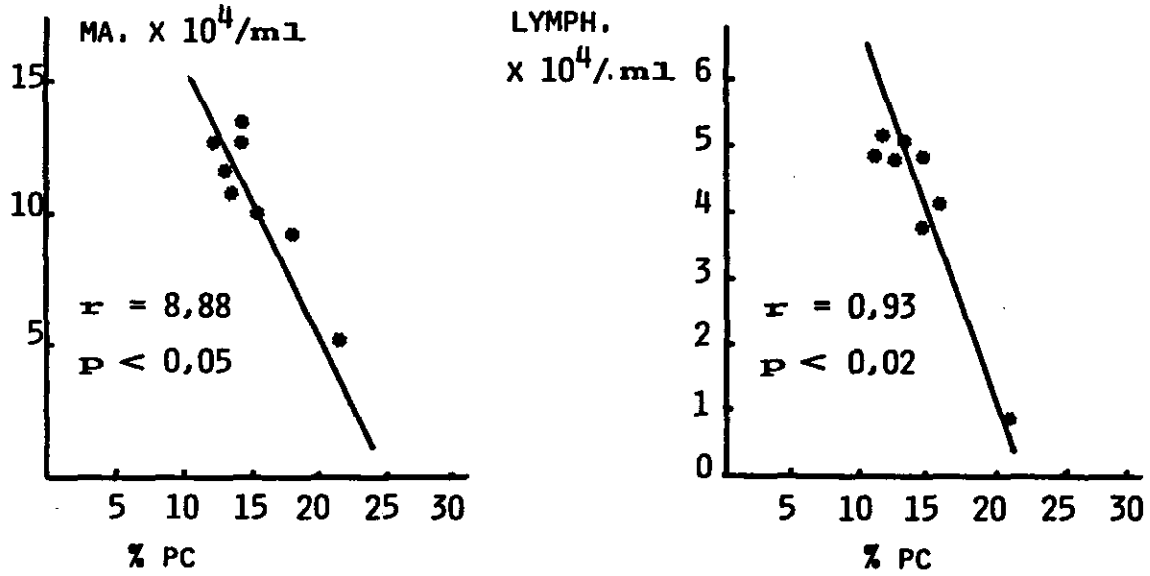
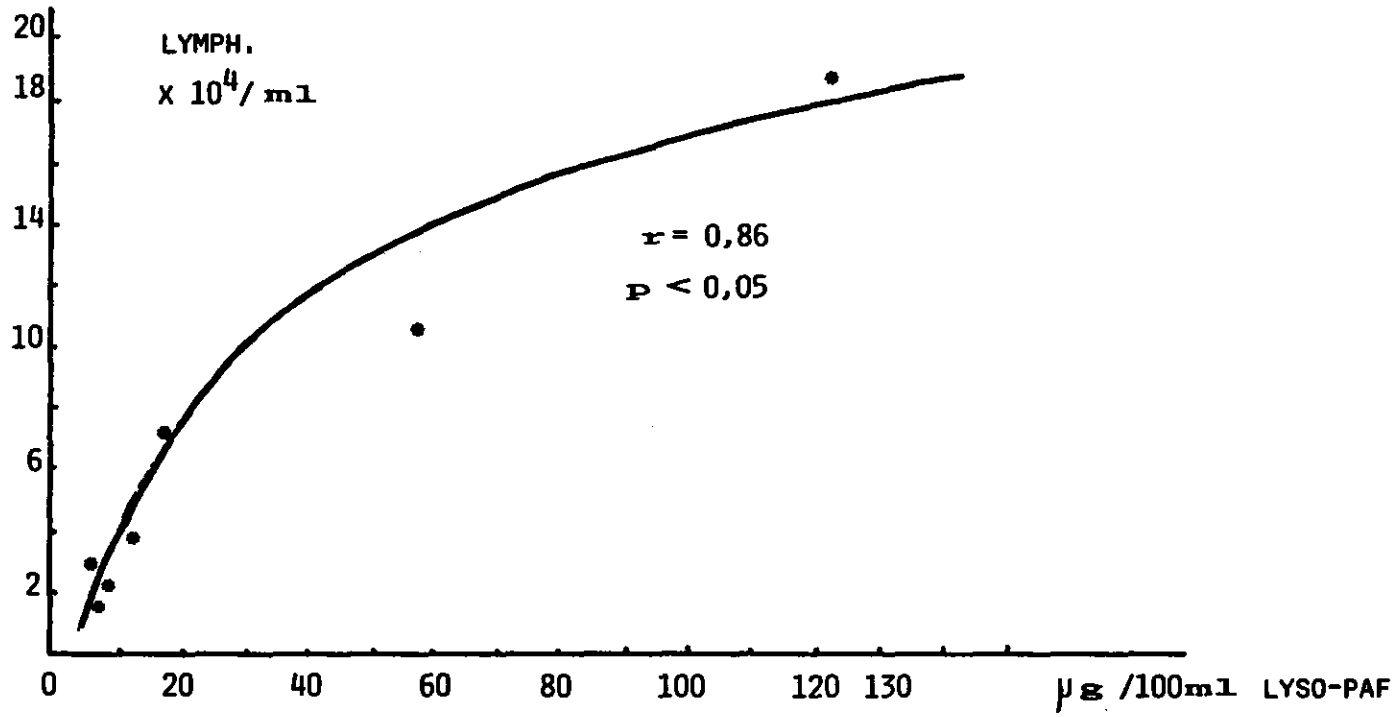


Table VIII



tion with the number of lavage lymphocytes. This hypothesis is reinforced by our previous demonstration that the A.M. of patients with occupational diseases caused by mineral containing dust releases, in vitro, Paf-acether when stimulated by the responsible dust through a mechanism uncompletely understood.[15] However we think that the above mentioned mechanism of perpetuation of the A.M. activation plays an important role in the synthesis of this lipid mediator.

Once admitted that in silicosis there is, in vivo, a chronic secretion of Paf-acether certainly it will play a role in the clinical picture of these patients either through its proinflammatory or bronchoconstrictive effects.[1,18]

In conclusion in silicosis there are, almost constantly, modifications in the composition of the lower respiratory tract fluid and the degree of alveolitis correlate reasonably with the gravity of disease, the complaints and the functional defects.

Moreover from the obtained data arises the central role of perpetually activated alveolar macrophage in Silicosis pathogenesis, either through the secretion of mediators, either to it ability to modulate other cells. Among these ones emerges the T-Lymphocyte and its subclasses importance. Finally the role of type II Pneumocyte must be referred due to its function of surfactant secreting cell.

REFERENCES

1. ARNOUX B.; Liberation et Activate Physiologique du Pat-Acether au Niviau de l'Appareil Pulmonaire; *These*, Paris (1987).
2. BENVENISTE J., Chignard M., Vargaftig BB; *Thromb Res.*, 25:375-386 (1982).
3. BEGIN R., Cartin A.M., Boileau R.D., Bisson G.Y.: Spectrum of Alveolitis in Quartz—Exposed Human Subjects; *Chest*, 92:1061-1067 (1987).
4. BEGIN R., Masse S., Sebastien P., Martel M., Bosse J., Dubois F., Sustained Efficacy of Aluminium to Reduce Quartz Toxicity in the Lung; *Exp. Lung Res.* 13:205-222 (1987).
5. CALHOUM W.J., Christman J.W., Ershler W.B., Graham W.G.B., Davis R.S.; Raisid Immunoglobulin Concentrations in Bronchoalveolar Lavage Fluid of Healthy Granite Workers; *Thorax* 1986; 41:266-273.
6. CHRISTMAN J.W., Emirson R.J., Graham W.G.B., Davis G.S.; Mineral Dust and Cill Ricovery from the Bronchoalveolar Lavage of Healthy Vermont Granite Workers; *Am. Rev. Respir. Dis.;* 132:393-399 (1987).
7. DAVIS G.S.; Pathogenesis of Silicosis: Current Concepts and Hypothesis; *Lung* 164:139-154 (1985).
8. ILO/UC International Classification of Radiographs of Pneumoconiosis 1980 No 22 Revised. Occupational Safety and Health Siriis. Genive: *International Labour Office*, 1980.
9. LOW R.B., Davis G., Giancola M.S.; Biochemical analysis of Bronchoalveolar Lavage Fluids of Normal Healthy Volunteers; *Am. Rev. Resp. Dis.* 118:863-876 (1978).
10. LUGANO E.M., Dauber J.H., Elias J.A., Bashey R.I., Jimenez S.A., Daniele R.D.: The Regulation of Lung Fibroblast Proliferation by Alveolar Macrophage in Experimental Silicosis; *Am. Rev. Resp. Dis.:* 129:761-767 (1984).
11. REYNOLDS H.Y., Chretien J.; Respiratory Tract Fluids: Analysis of Content and Contemporary Use in Understanding Lung Diseases. *Disease-a-Month Year Book Medical Publishers*, Vol. 30, No 5, 1984.
12. SCHWARZ M.I., King Talmadge E.; Interstitial Lung Disease; *B.C. Decker Inc.*, pag. 63-109.
13. SCHUYTER M.R., Gaume H.R., Stankus R.P., Kaimal J., Hoffman E., Salvaggio J.E.; Bronchoalveolar Lavage in Silicosis; *Lung* 157:95-102 (1980).
14. SIMÕES M.H., Teles de Araujo A., Arnoux B., Palma-Carlos A.G., Freitas e Costa M., Benveniste J.; The Release of Paf-acether and Lyso-Paf-acether Bronchoconstrictive Mediators by Alveolar Macrophages on Granulomatosis. Proceedings of the IV European Conference on Sarcoidosis and Other Granulomatous Disorders; *Ed. Blasi, Olivieri; Pezza* pag. 229-234 (1983).
15. TELES DE ARAÚJO A., Alfarroba E., Freitas e Costa M.; The Role of Monoclonal Antibodies in the Study of Chronic Inflammatory Diseases Induced by Dust Inhalation; *Eur. J. Resp. Dis.* Sup1.146, 146:203-210 (1986).
16. TELES DE ARAÚJO A., Mendes Ana, Monteiro J. Tomás, Freitas e Costa M.—Characteristic of the Lymphocytary Alveolitis of Sarcoidosis and Mineral Dust Inhalation Induced Pulmonary Granulomatosis. Sarcoidosis and Other Granulomatous Disorders; *Elsevier Science Publishers*, 711-712 (1988).
17. ROUBIN R., Tence M., Mencia-Huerta J.M., Arnoux B., Nínio E., Benveniste J.; A Chemically Defined Monokine: Macrophage Derived Platelet-Activating Factor (Paf-Acether); *Lymphokines*, Vol. 8, pag. 249-276 (1983).
18. VAN GOLDE L.M.G.; Synthesis of Surfactant Lipids in the Adult and Fetal Lung: Pathways and regulatory aspects; *European Journal of Respiratory Diseases;* Supl. 142; Vol. 67, pag. 19-28 11986).
19. WHICHEST P. Endobronchial Surface Active Phospholipids: Clinical Conclusions; *European Journal of Respiratory Diseases;* Supl. 142, Vol. 67, pag. 77-78 (1985).

The Paf-Acether, Lyso-Paf-Acether and correlated Etherlipids assays were performed in the Unite 200 de l'INSERM, Clamart, France (Director Dr. J. Benveniste) with a grant provided by the "Accord Culturelle Scientifique et Techenique entre le Portugal et la France."

INHALED CORTICOSTEROIDS IN THE TREATMENT OF OCCUPATIONAL RESPIRATORY DISEASES (O.R.D.)

J. ROSAL GONCALVES • Isabel Correia • Elena Raymundo • A. Teles de Araújo • M. Freitas e Costa

Clínica de Doencas Pulmonares (FML) and CnL3-INIC-Lisboa-Portugal

INTRODUCTION

The Occupational Respiratory Diseases (O.R.D.) are nosological entities for which it is possible to find a relationship between the dust, gas and aerosols inhalation in the work environment and the disease emergence.

The O.R.D. can display as an interstitial lung disease or a chronic air-flow obstruction. Frequently, the two syndromes overlap[1,2,3,8] and the basic pathogenic mechanisms are similar in both cases depending upon the clinical differences in the characteristics of the inhaled noxious substance and the individual behaviour.

In the pathogenic pathways of O.R.D. the alveolar macrophage fulfils an important role. Its stimulation releases Il_1, activating the T-lymphocyte with release of Il_2, peripheral monocyte recruitment, local T cell proliferation and a B cell stimulation leading to the granuloma formation.[5]

The perturbation of the macrophage cell membrane by stimulus, antigenic or others, causes the activation of the Phospholipase A2, interfering with the membrane phospholipids and leading to the release of arachidonic acid molecules and its metabolites.[11,14] The release of Paf-acether by the alveolar macrophages of these patients,[10,14] and of toxic 02 species has also been demonstrated.

The activated alveolar macrophage also releases fibronectin and Macrophage Derivated Growth Factor, important mediators in the fibrotic process.[1,3,8]

From the destruction of the alveolar macrophage by the cytotoxity of the noxious substance and from incomplete lysosomes results the release of enzymes—elastase and collagenase—contributors to the interstitial lung damage.[8,11]

Also the neutrophils are increased in the alveolar spaces and when stimulated by immune complexes liberate noxious enzymes.[8,11]

Finally in O.R.D. patients, as it happens in other fibrotic diseases, there is an increased number of mastocytes in the interstitial spaces and the released histamine would perhaps have a proinflammatory effect besides its bronchoconstrictive action.[13,15]

From the above mentioned emerges the justification to the use of corticosteroids in the treatment of some O.R.D. patients through its capacity to blockade the interleukins and other mediators release, to inhibit the Phospholipase A2, to diminish the neutrophils adhesivity and chemotaxie and to inhibit the production of histamine.

In clinical trials we had already confirmed that the improvement of the O.R.D. patients under corticotherapy is accompanied by a significant diminishing of the number of T-lymphocytes and rates of Lyso-Paf-acether (the percursor of Paf-acether) and histamine.[13,14]

As the local of the pathogenic process is the epithelial alveolar surface, it seems reasonable to think that the inhaled corticosteroids could perhaps stop them and be useful in the treatment of these O.R.D. patients requiring the use of drugs for their management.

The aim of this study is to evaluate the effectiveness of the inhaled corticosteroids in the treatment of the occupational respiratory diseases.

PATIENTS AND METHODS

We have studied 15 patients with ages ranging between 28 and 66 years, mean age 49 years. Ten of the patients were males and 5 females and two of the men were smokers (Table I).

Nine of the patients had a consistent occupational history of exposition to mineral dusts (5 to silica, 3 to iron and 1 to asbestos) and 6 to organic dust (3 to corck dust and 3 to pigeon dregs)—Table II.

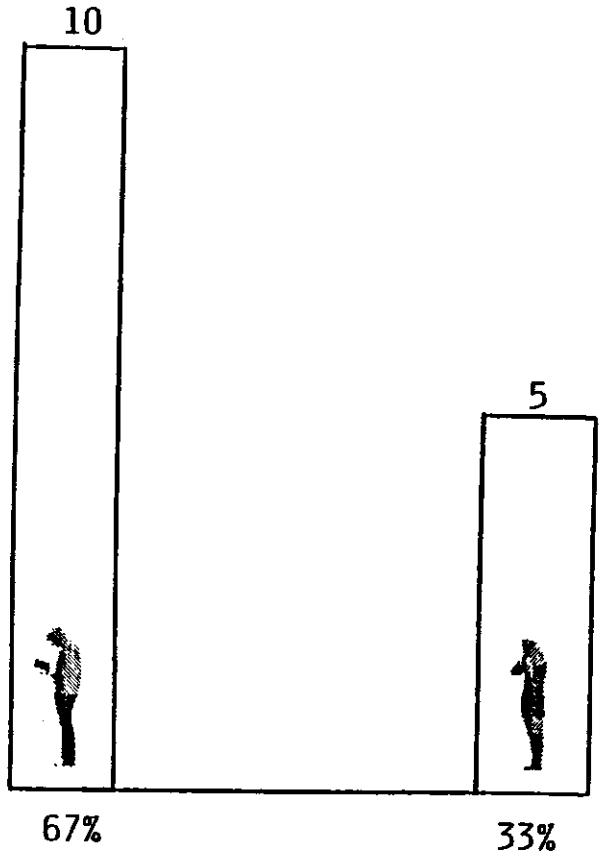
All of them had the disease confirmed through the usual clinical, functional, immunological and histological criteria.

All the patients had been submitted to a clinical inquiry, a standard chest X-ray and a complete functional respiratory study (global body plethysmography) previously to the treatment. The same study has been repeated every three months during one year.

The patients without evidence of ventilatory obstruction were submitted to bronchoalveolar lavage in a subsegment of the middle lobe with 4 syringes of 50 ml of saline serum warmed up to 37°C by a usual technique.[1] In 3 of the patients the bronchoalveolar lavage has been repeated 6 months after the beginning of the treatment.

The patients were submitted to a therapeutic with Budesonide 4 × 200 μg twice a day, via a 750 ml spacer.

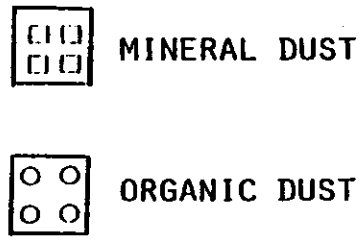
Table I
Patients

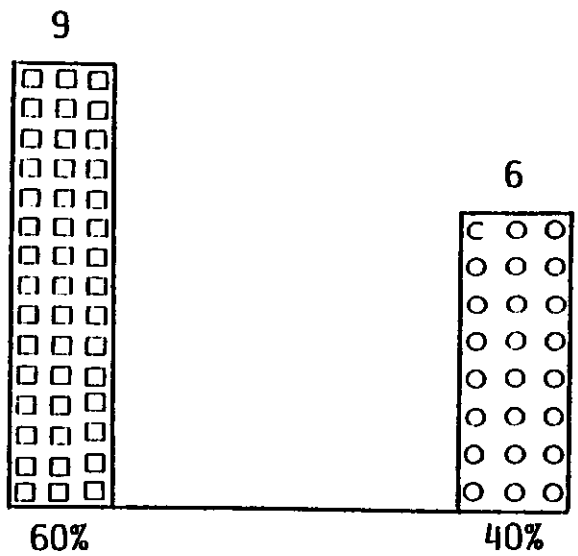


TOTAL - 15

MEAN AGE - 49
MAX. " - 66
MIN. " - 28

Table II
Exposition





As concomitant medication we used in 7 patients inhaled bronchodilators. None of the patients had concomitant or previously oral corticosteroids. All were kept away from their workplace.

As comparison terms we considered a control group of 5 other O.R.D. patients with similar clinical, radiological and functional patterns, also kept away from the workplace in which only oral bronchodilators had been prescribed.

For statistic analysis we used the T test for paired data, differences method.

RESULTS

Before treatment all patients had complaints of exertion dyspnoea, 14 (93%) had cough, 8 (53%) expectoration and 7 (46%) wheezing (Table III).

After the first 3 months of treatment the clinical evaluation stated an improvement of the complaints in 12 (80%) of the patients, increasing progressively throughout the complete period of study.

The only side effects reported were two cases of mild sore throats and one of hoarseness, not being necessary to stop treatment.

In the control group only 3 out of the 5 patients (60%) improved.

All patients of both groups had chest X-ray before treatment evocating interstitial lung involvement expressed by linear and round shadows classifiable as, at least, of the type p 1/1 (UICC/Cincinnati classification). These aspects did not modify throughout the period of the study. However, in three patients with confluent shadows this aspect disappeared during the treatment.

At the beginning of the treatment 4 of the patients had functional obstructive defects, six restrictive defects and 5 had a normal pattern.

From the observation of Table IV it is clear that the Vital Capacity improved during the treatment in 12 of the patients (80%) and from the 3 that did not improve 2 had previously normal values. This improvement is significant ($p < 0.001$).

In what concerns the Total Lung Capacity only 9 of the patients improved (60%) and the difference is not significant (Table IV).

In Table IV the FEV_1 values are analyzed and it is verifiable that there is a significant improvement during the treatment ($p < 0.05$).

On the contrary the Tiffeneau index did not modify significantly with the treatment (Table IV).

In the control group there is no significant modification in any of the studied parameter (Table V).

The eight patients without evidence of bronchial obstruction were submitted to bronchoalveolar lavage. In 4 of them we verified the existence of an alveolitis ($74.5 \pm 71.3 \times 10^4$ cells/ml) and in both groups a significant increase of the lymphocytes percentages—three folds the normal values—(Table VI).

All patients of this group referred improvement in complaints during the treatment and ventilatorily and a significant increase in the values of Total Lung Capacity and Vital Capacity has been found (Table VII).

The three patients in which a second lavage had been performed showed 6 months after the beginning of the treatment, a decrease in the total cell count and in the number of lymphocytes as it is demonstrated in Table VIII.

DISCUSSION

First we must emphasize the difficulty to take conclusions from such a heterogeneous population in what concerns the type of inhaled agressor and the clinical manifestations.

By obvious reasons in a preliminary study we had chosen patients with a relatively mild disease, in which a sufficient

Table III

Complaints

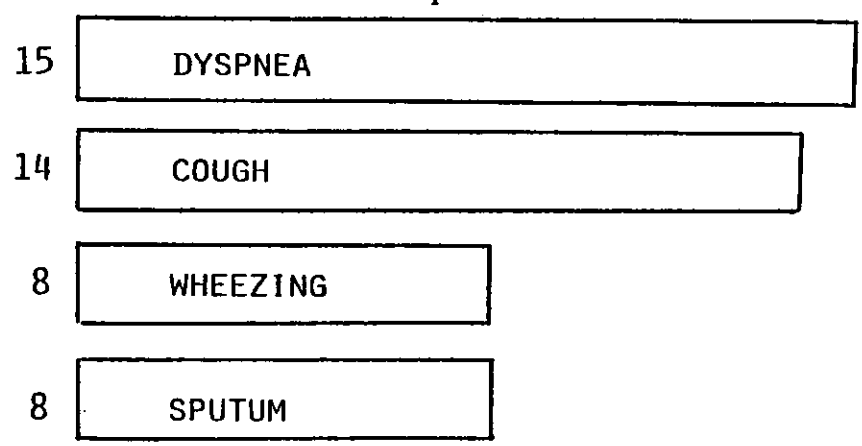
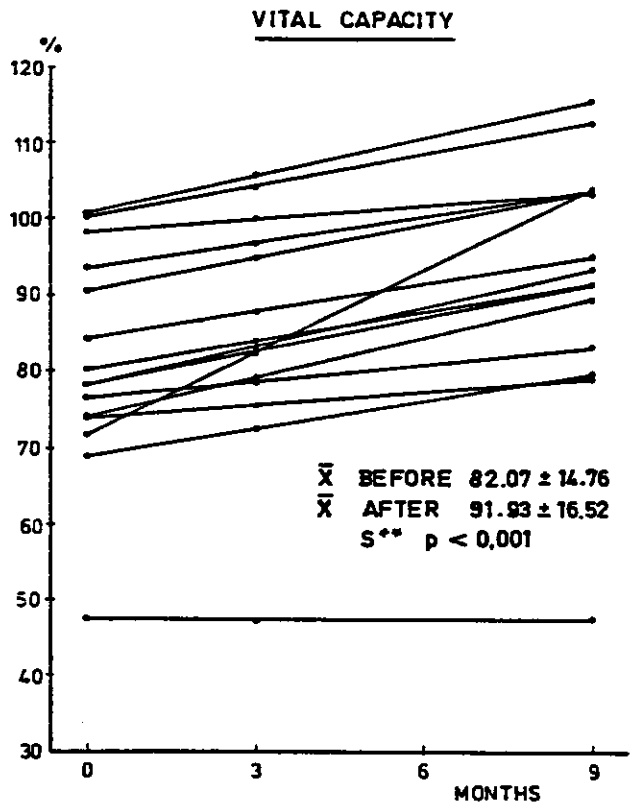
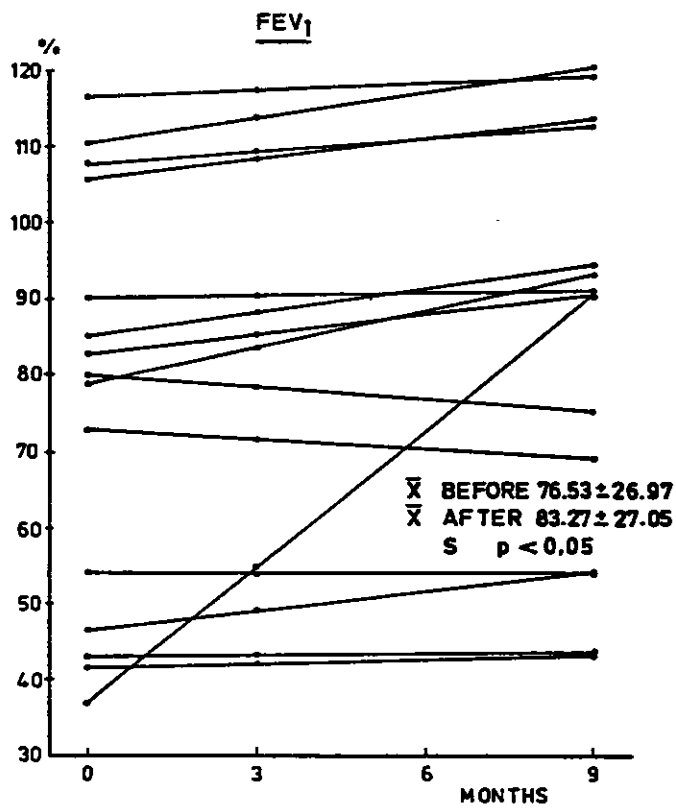
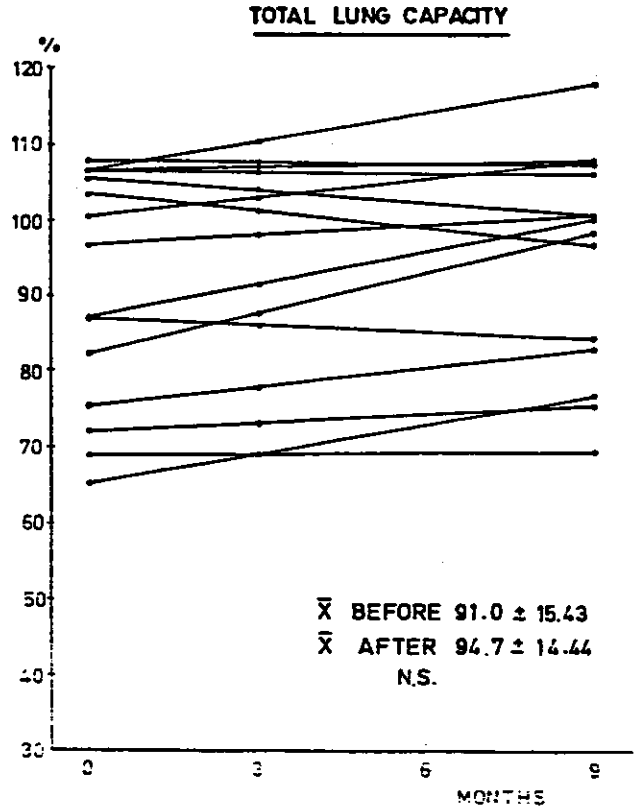


Table IV


VITAL CAPACITY
%
120
110
100
90
80
70
60
50
40
30
0
3
6
9
MONTHS
X̄ BEFORE 82.07 ± 14.76
X̄ AFTER 91.93 ± 16.52
S** p < 0,001


FEV1
%
120
110
100
90
80
70
60
50
40
30
0
3
6
9
MONTHS
X̄ BEFORE 76.53 ± 26.97
X̄ AFTER 83.27 ± 27.05
S p < 0,05


TOTAL LUNG CAPACITY
%
120
110
100
90
80
70
60
50
40
30
0
3
6
9
MONTHS
X̄ BEFORE 91.0 ± 15.43
X̄ AFTER 94.7 ± 14.44
N.S.

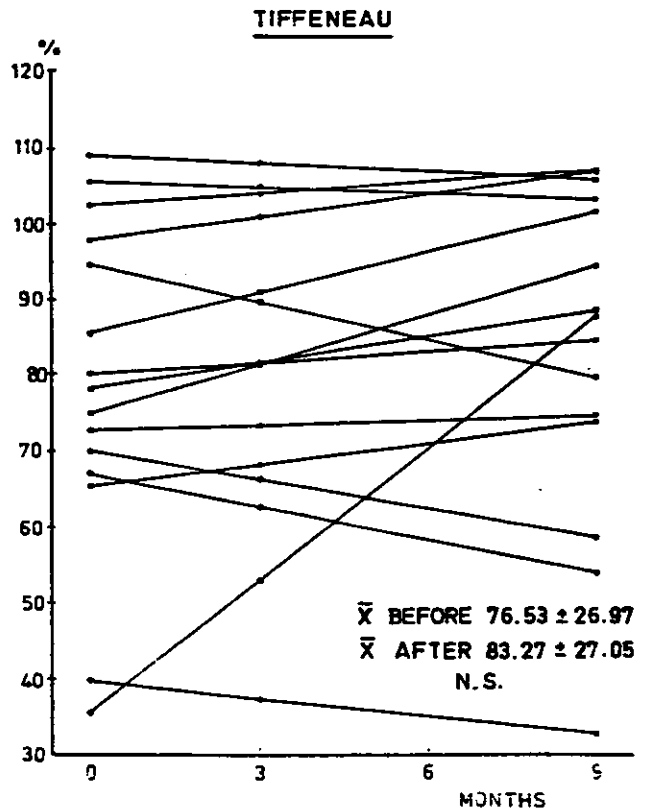
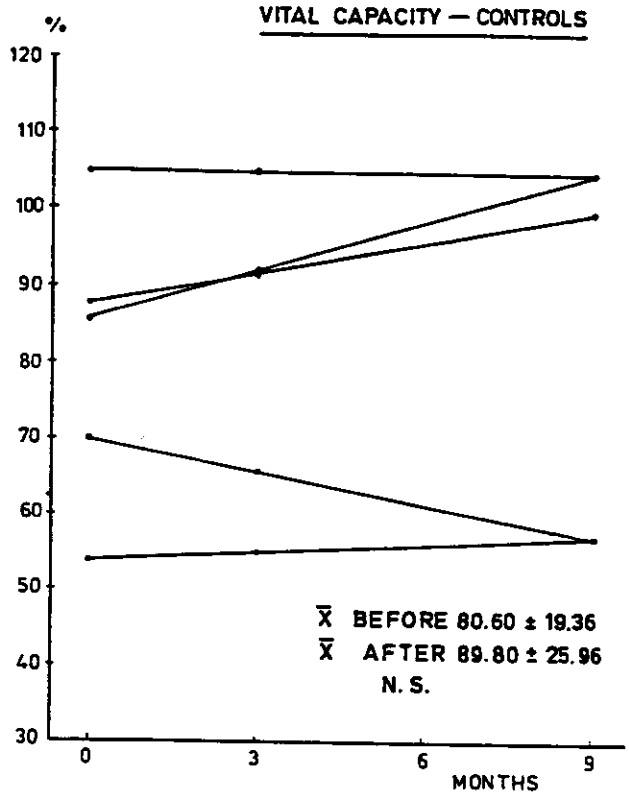
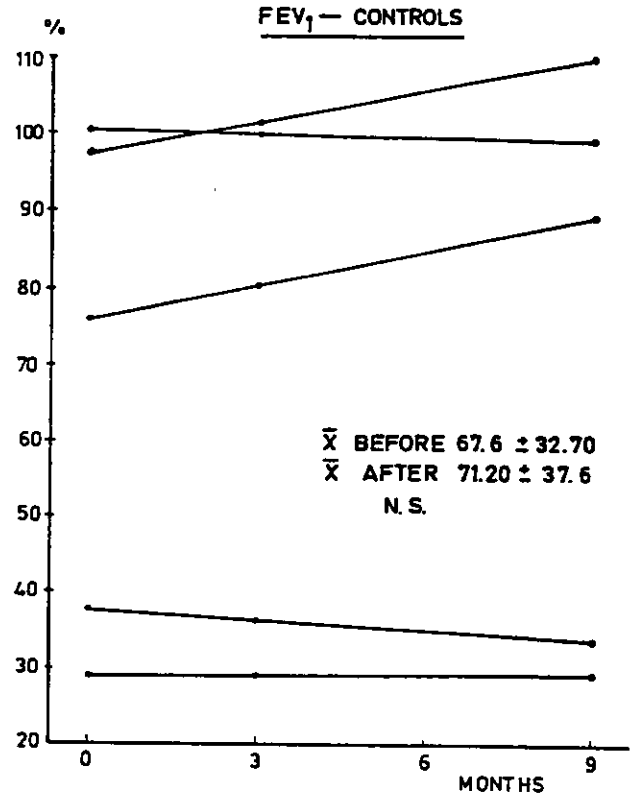
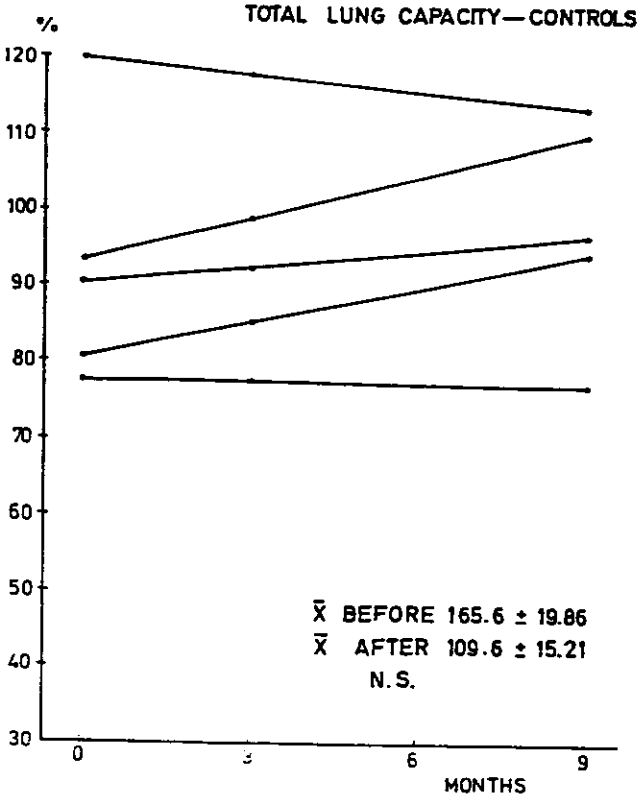
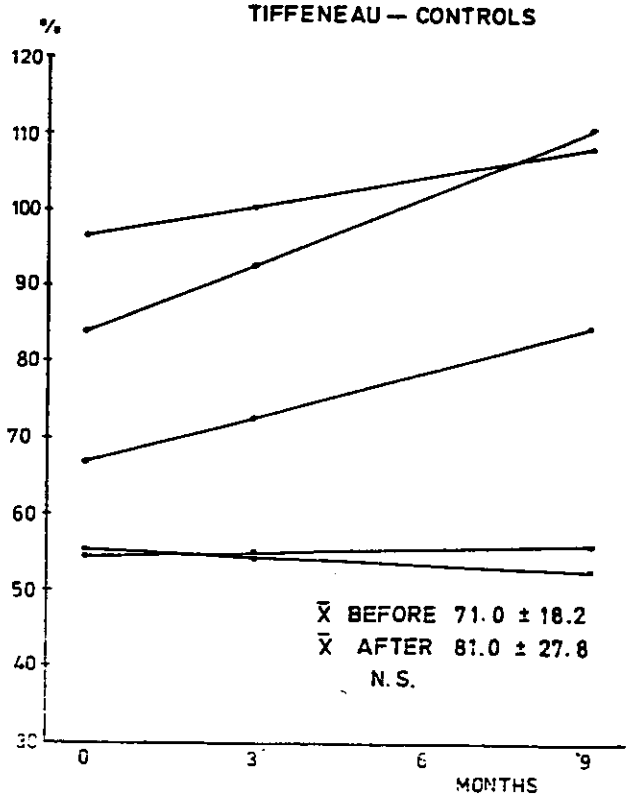

TIFFENEAU
%
120
110
100
90
80
70
60
50
40
30
0
3
6
9
MONTHS
X̄ BEFORE 76.53 ± 26.97
X̄ AFTER 83.27 ± 27.05
N.S.

Table V


VITAL CAPACITY — CONTROLS
%
120
110
100
90
80
70
60
50
40
30
0
3
6
9
MONTHS
X̄ BEFORE 80.60 ± 19.36
X̄ AFTER 89.80 ± 25.96
N. S.


FEV1 — CONTROLS
%
110
100
90
80
70
60
50
40
30
20
0
3
6
9
MONTHS
X̄ BEFORE 67.6 ± 32.70
X̄ AFTER 71.20 ± 37.6
N. S.


TOTAL LUNG CAPACITY—CONTROLS
%
120
110
100
90
80
70
60
50
40
30
0
6
9
MONTHS
X̄ BEFORE 165.6 ± 19.86
X̄ AFTER 109.6 ± 15.21
N. S.


TIFFENEAU — CONTROLS
%
120
110
100
90
80
70
60
50
40
0
3
6
9
MONTHS
X̄ BEFORE 71.0 ± 18.2
X̄ AFTER 81.0 ± 27.8
N. S.

clinical, radiological or functional support of an interstitial involvement had been found; however, this group included an important number of patients with simultaneous chronic airflow obstruction, sometimes even prevalent.

Supporting the favourable clinical evolution of the patients under inhaled corticotherapy the functional ventilatory parameters that more consistently improved were those related with the interstitial involvement rather than those related with airflow obstruction.

The suggestion of the interest of these drugs in O.R.D. patients is reinforced by the favourable clinical evaluation of the patients with alveolitis, confirmed by bronchoalveolar lavage (BAL) and by the improvement of the cellular parameters of their BAL fluid during the treatment.

The anarchical response of obstructive parameters to the therapeutic measures suggests the interference of other factors in bronchoconstriction independently of corticosteroids action: tobacco, infection, etc.

Besides the interest of inhaled corticosteroids in diseases with airflow obstruction[7,9] it has already been demonstrated in Sarcoidosis that the inhalatory therapy with Budesonide is able to transform the initial cellular, biochemical and immunological abnormalities in the direction of normalization and that the clinically useful doses result in tissue concentrations high enough to be efficacious.[9]

These two perspectives are very important in O.R.D. patients.

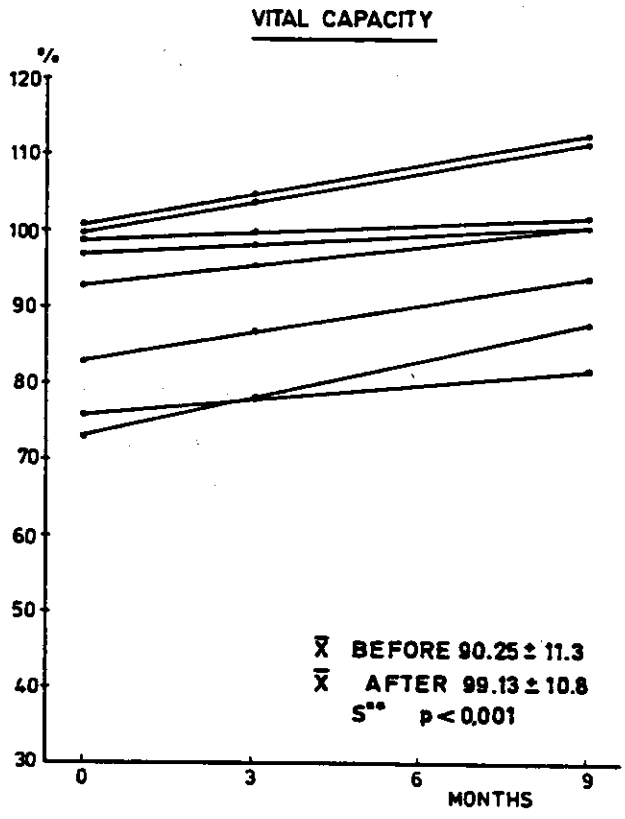
In fact a drug sufficiently efficient to reach the alveoli and stop the release of mediators by the immunological and inflammatory effector cells, and simultaneously to persist in the interstitium braking the pathogenic mechanisms due to the persistence of the aggressive particle, would surely have a place in the management of these diseases.

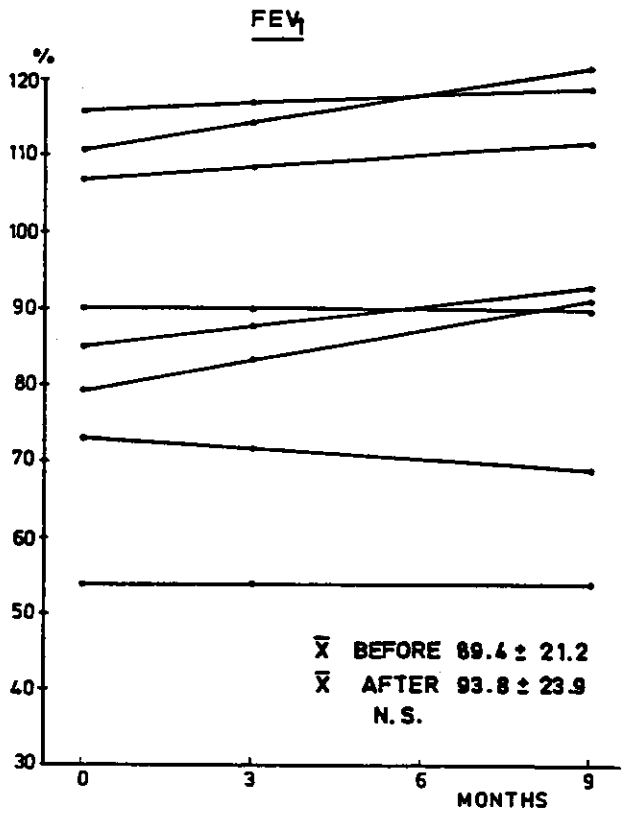
The obtained results seem to provide some evidence of the effectiveness of the purposed treatment, mainly in the interstitial occupational respiratory diseases which is not surprising once admitted the pathogenic mechanisms described above.

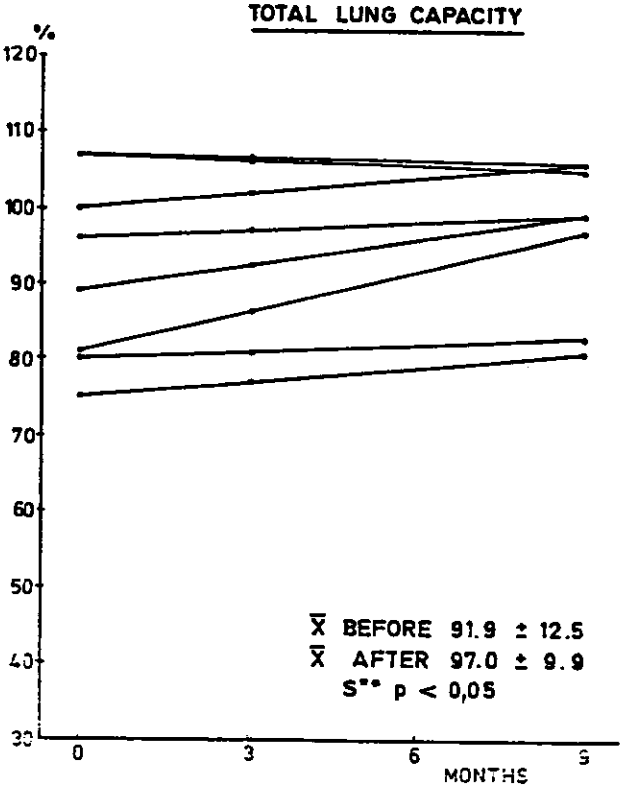
Table VI
Bronchoalveolar Lavage

	CELLS	MA	LYMPH.	P.M.N.
WITH ALVEOLITIS n = 4	74,5±71,3	53,8±32,5	39,5±23,2	1,75±1,5
WITHOUT ALVEOLITIS n = 4	18,3± 6,9	63,3±28,6	36,3±28,6	1,0 ±1,4

Table VII
Patients with B.A.L.







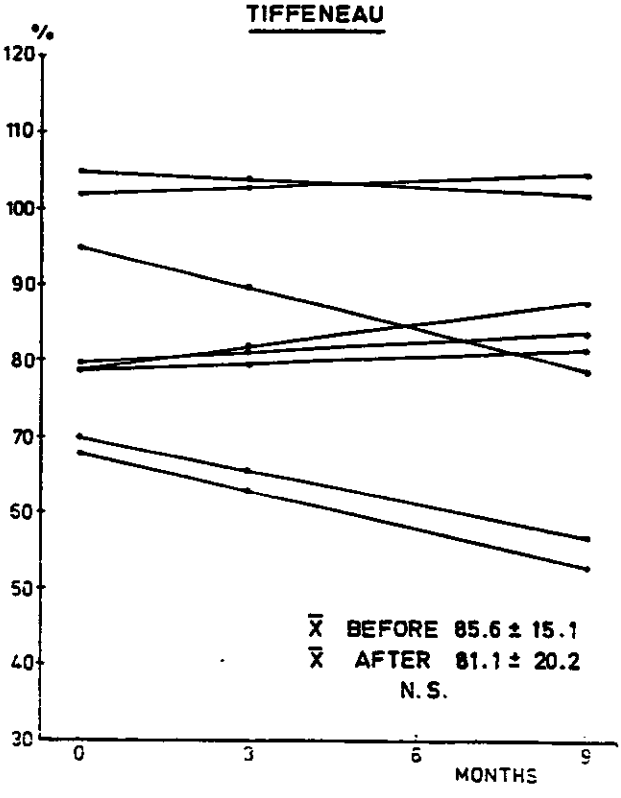
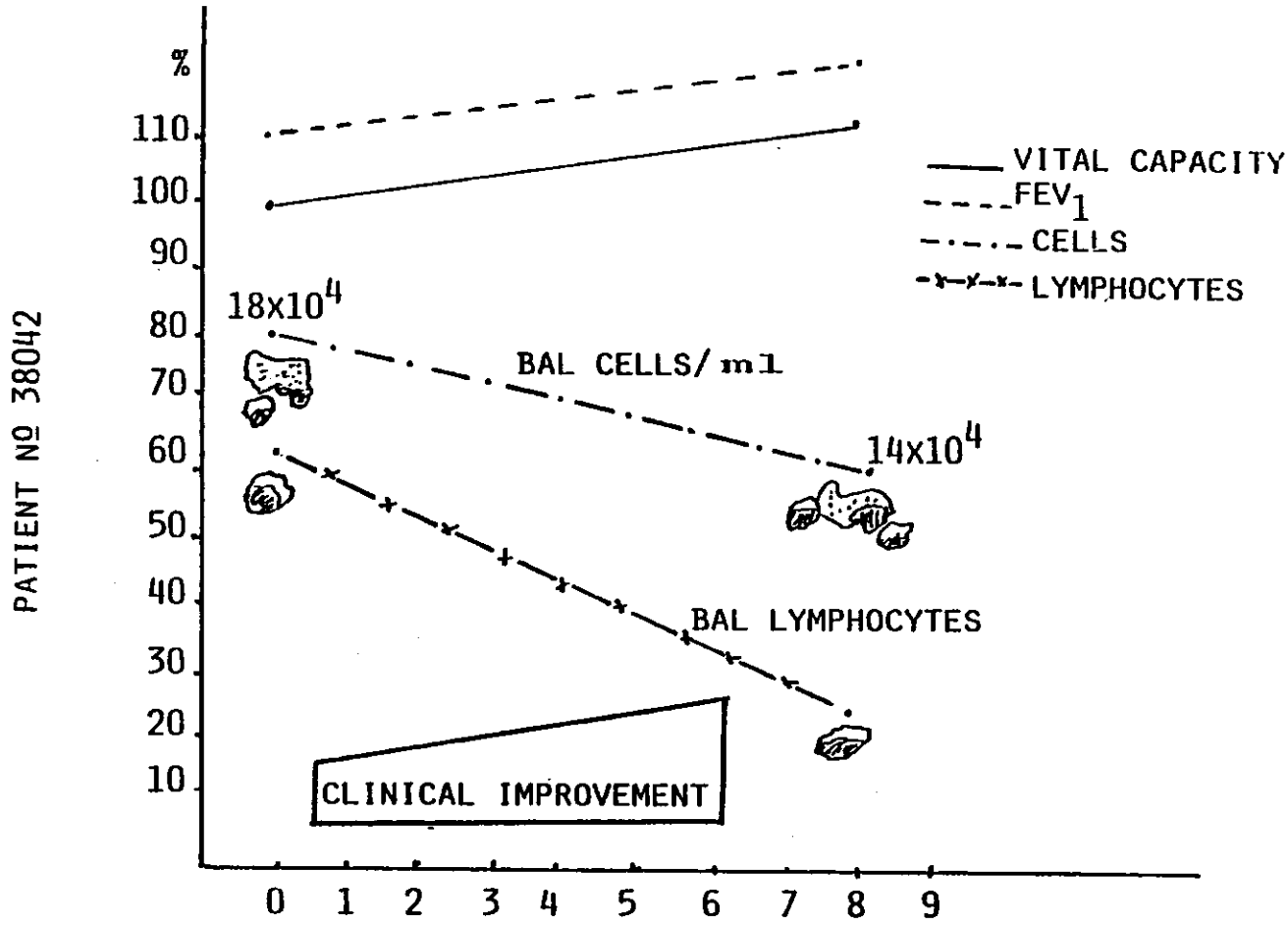


Table VIII



Thus when it is necessary to consider the use of corticosteroids the inhalatory therapy can represent an alternative or a complement to oral corticosteroids. This alternative becomes more important if one considers that inhaled corticosteroids rarely cause systemic side effects and do not reach immunosuppressive levels,[2] which is of a great interest in patients with susceptibility to infections such as the case of Silicosis.

Further studies will be necessary to define the real usefulness of Budesonide in the treatment of these patients, which are the parameters necessary to define when it can be used as the unique therapeutic measure, besides the evication from the aggressive noxious, and when it must be used as a complement to oral corticosteroids, permitting a significant dose reduction.

REFERENCES

1. Bernard, J., Gree, L.: Occupational Lung Diseases. Contemporary Issues in Pulmonary Diseases (1984).
2. Brooks, Stuart M., Kalica, Anthony, R.: Strategies for Elucidating the Relationship Between Occupational Exposures and Chronic Airflow Obstruction (NHLBI Workshop Summary). *Am. Rev. Resp. Dis.* 135:268-273 (1987).
3. Dunnil, M.S.: Pulmonary Pathology. Interstitial Lung Diseases. *Ed. Churchill Livingston*, pag. 487-529 (1987).
4. Haslam, P.L., Cromwell, O., Dewar, A., Turner-Warwick: Evidence of Increased Histamine Levels in Lung Lavage Fluids from Patients with Cryptogenic Fibrosing Alveolitis. *Clin. Exp. Immunol.* 44:587-593 (1981).
5. Hunninghake, G.W., Ganett, K.C., Richardson, H.B., Fantone, J.C., Ward, P.A., Rennard, S.I., Bitterman, P.B., Crystal, B.G.: Pathogenesis of the Granulomatous Lung Diseases. *Am. Rev. Resp. Dis.* 130:476-496 (1984).
6. Lugano, E.M., Dauber, J.H., Elias, J.A., Bashey, R.I., Jimenez, S.A., Daniele, R.P.: The Regulation of Lung Fibroblast Proliferation by Alveolar Macrophages in Experimental Silicosis. *Am. Rev. Resp. Dis.* 129:767-771 (1984).
7. Reynolds, H.Y., Chrétien, J.: Respiratory Tract Fluids: Analysis of Content and Contemporary Use in Understanding Lung Diseases. *Disease-a-Month. Year Book Medical Publishers, Inc.* Chicago, February, (1984).
8. Schwarz, M.I., King, T.E.: Interstitial Lung Disease. *B.C. Decker*, Inc., 63-110 (1988).
9. Selroos, O.: Use of Budesonide in the Treatment of Pulmonary Sarcoidosis. Advances in the Use of Inhaled Corticosteroids Symposium. *Excerpta Medica*, (1986).
10. Simões, M.H., Araújo, A.T., Arnoux, B., Palma-Carlos, A.G., Freitas e Costa, M., Benveniste, J.: The Release of Paf-acether and Lyso-Paf-acether Bronchoconstrictive Mediators by Alveolar Macrophages on Granulomatosis. Proceeding of the IV European Conference on Sarcoidosis and Other Granulomatous Disorders. *Ed. Blasi, Olivieri, Pezza*, pag. 229-234 (1983).
11. Taylor, A.Y.N.: The Lung and the Work Environment. Current Perspectives in the Immunology of Respiratory Disease. *Ed. A.B. Kay, Edward, Y. Greetz, Churchill Livingston*, pag. 128-142 (1988).

12. Teles de Araujo, A., Alfarroba, E., Freitas e Costa, M.: The Role of Monoclonal Antibodies in the Study of Chronic Inflammatory Diseases Induced by Dust Inhalatory. *Eur. Y. Resp. Dis. Supl.* 146:203-210 (1986).
13. Teles de Araujo, A., Mendes, A. Cristina, Monteiro, J.T., Freitas e Costa, M.: Characteristics of the Lymphocytary Alveolitis of Sarcoidosis and Mineral Dust Inhalation Induced Pulmonary Granulomatosis. *Sarcoidosis and Other Granulomatous Disorders.* 711-712 (1988).
14. Teles de Araujo, A., Reis Ferreira. J.M., Freitas e Costa. M., Benveniste. J.: Abnormalities of Lipid Composition of Bronchoalveolar Lavage (BAL) in Respiratory Diseases Induced by Dust Inhalation. Sarcoidosis and Other Granulomatous Disorders. *Ed. Elsevier Science Publishers,* 685-688 (1988).
15. Teles de Araujo, A., Valenca, J., Bugalho de Almeida, A., Freitas e Costa, M.: Release of Histamine by Cultures of Bronchoalveolar Adherent Cells, *Prog. Resp. Res.*, 19:35-42 (1985).
16. Tukrainen, P., Lahdensuo, A.: Effect of Inhaled Budesonide on Severe Steroid-Dependent Asthma. *Eur. J. Resp. Dis.* 70:239-244 (1987).

ANALYSIS OF FATTY ACIDS FRACTIONS OF PHOSPHOLIPIDS AND NEUTRAL LIPIDS FROM BRONCHOALVEOLAR LAVAGE FLUID (BALF) IN PATIENTS WITH OCCUPATIONAL LUNG DISEASES (OLD)

GINA DUARTE • Ema Fonseca • A. Teles de Araujo • M. Freitas e Costa

Centro de Investigacão CnL3(INIC) and Laboratório de Farmacologia
do Instituto Gulbenkian da Cência, Oeiras, Portugal

INTRODUCTION

The pulmonary surfactant has caught the attention of numberless investigators since its discovery was reported by Pattle[1] and the primary characteristics of the substance were first described by Clements.[2]

Basically the pulmonary surfactant reduces surface tension of the interface air-liquid of the alveolus, maintaining mechanical stability and avoiding alveolar collapse particularly at small pulmonary volumes.[3,4,5] Other functions of this complex substance are the help on removal of particles from airways and on digestion of bacterias intra and extra cellulars, the inhibition of pulmonary edema and the transudation of fluids into the alveolus.[7,8,9]

Dipalmitoyl phosphatidylcholine (DPPC) is the predominant molecule of the pulmonary surfactant and of it depends, in great part, the tensio-active function of this compound.[3,5,6] The exact functions of the remaining components such as insaturated phosphatidylcholine, other phospholipids, neutral lipids, and specific proteins are incompletely studied.[3,10]

The pulmonary surfactant is produced in the pneumocyte type II, synthetized in its endoplasmic reticular system and transferred, by mechanisms not fully known, to the lamellar bodies and then secreted to the alveolar surface.[3,11]

Several enzymatic mechanisms are involved in the synthesis of phospholipids and there is evidence of a certain hormonal regulation—androgenes, corticosteroids, tiroxine and insulin.[11,3]

In the control of the secretion there seem to be involved cholinergic and B adrenergyc mechanisms. Chemical mediators such as prostagl; dines, physical factors such as the distortion of the alveolar membrane and hyperventilation can also interfere in surfactant secretion.[11,12]

The importance of surfactant defects is well established in the pathogenesis of diseases like neonatal and adult respiratory distress syndrome[13,14] and less well established in other situations of respiratory suffering in man.[5,6]

Since on occupational lung diseases there are important immunologic and inflammatory mechanisms occurring in the surface of the alveolus including changes in the alveolar microatmosphere, thickness and rigidity of the membranes and distortion of the structure,[15,16,17] an hypothesis was sought that this factor could condition changes in the composition of the pulmonary surfactant.

In order to attempt to prove it, we studied the lipidic composition—phospholipids, neutral lipids and fatty acids—of the extracellular compartment of the pulmonary surfactant, obtained through bronchoalveolar lavage in 15 individuals: 5 normal, 5 pigeon breeders and 5 silicosis patients.

MATERIAL AND METHODS

Patients

We studied 5 patients with silicosis and 5 with pigeon breeder's lung, diseases confirmed through usual criteria.

Both groups were homogeneous concerning age and sex. None of the patients smoked cigarettes and none was submitted to corticotherapy.

All were submitted to a clinical and functional study, a standard thorax X-ray and bronchoalveolar lavage.

As controls we used 5 normal volunteers, non-smokers with ages not differing significantly of those of the patients.

Bronchoalveolar Lavage

All individuals studied were submitted to BAL in one of the sub-segments of the middle lobe. Four fractions of 50 ml of saline at 37°C were instilled with a syringe adapted to the fibroscope and after a few seconds the liquid was retrieved. The mucus was separated, total cell count was performed by hemocitometry. The cellular component was separated by centrifugation at 500 G for 15 min. at 4°C. The supernatant was dried in a vacuum oven −50°C to −60°C for several hours and stored in a steam of nitrogen for further study.

PROCESSING AND ANALYSIS OF LIPIDS

Extraction of Lipids

The total lipids were extracted from the dried supernatant lavage fluid according to Bligh and Dyer method.[25]

Chromatographic Methods

Total lipids were separated by column chromatography on silica acid-Kieselguhr (BDH), in neutral lipids (NL) and phospholipids according to Cmelik and Fonseca.[26]

The neutral lipids (cholesterol, cholesterol esthers fatty acids and triglycerids fractions) were separated by thin layer chromatography (TLC) on silica gel G plates (Merck Chemical Co.) using petroleum ether/ethyl/acetic acid (70:30:1) as developer.

Phospholipids were separated by TLC on silica gel H (Merck) and Florisil (BDH 10-200 mesh) plates using chloroform/methanol/water (65:25:4) as a solvent.

NL fractions were hydrolyzed with a 5% potassium metoxide solution. After the extraction of the non-saponifiable part, fatty acids were extracted from the acidified solution with ethyl ether.

Phospholipids were hydrolyzed with HCl 6N in sealed tubes immersed in a boiling water bath for 4-6h.

Fatty acids were converted into methyl esters with a methanolic solution of Boron trifluoride BF_3[27] and analyzed on a Perkin Elmer 900 gas-chromatograph with a dual flame ionization detector. The columns were 2 m long with an 1/8 inch o.d. and were packed with 20% Diethylenoglycol Succinate (DEGS) and chromosorb W (DMCS) 80–100 mesh. The analysis was performed at programmed temperature (140°–170°C) with an increasing rate of 2°C minute, followed by isothermal operation. The nitrogen flow rate was 35 ml/min. Peak's identification was determined by comparing their relative retention time with that of known standards. The relative percentage of the peak areas was evaluated by an integrator from Hewlett Packard's 3.380 A).

Spots on analytical plates were visualized by spraying with concentrated sulfuric acid containing 0.1% of potassium dichromate and subsequent charring at 140°C. Spots of the phospholipids were identified by the use of respective standards and phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were identified too by spraying the plates with Dragendorff and Ninhydrin reagents respectively.

Total cholesterol was determined by the use of a modified Lieberman-Buchard reagent.[28] Phosphorus analysis was performed by the method of Fiske and Subbarow[29] as modified by King.[30]

Total proteins were assayed by Lowry's method.[31]

RESULTS

Study of phospholipids revealed some differences on both groups of patients in relation with controls (Table I). Therefore on pigeon breeders a significant decrease of phosphatidylcholine was found as well as phosphatidylglycerol on silicosis patients.

On the other hand, in both groups we found significant increases of phosphatidylethanolamine and sphingomyelin.

As for neutral lipids, the fact that cholesterol was abundant in the 3 groups prevailed. Also in the 3 groups free fatty acids were detected (Table II).

In the composition on fatty acids of phospholipids, significant differences on both groups of patients were also detected by comparison with the controls (Table III), mainly the important decrease of C16:0 and increase of fatty acids insaturated in C16, C18 and C20. To be noted also the significant increase of arachidonic acid on pigeon breeders. From this results that the ratio between saturated and insaturated fatty acids is inferior to unity on patients and superior to 2 on controls (Table IV).

From the analysis of the composition in fatty acids of the neutral lipids once more highlights the important decrease of palmitic acid and the increase of insaturated fatty acids in patients, mainly in C16 and C18 (Table V). The ratio saturated/insaturated fatty acids results once again less than unity on patients and greater than 2 on controls (Table VI).

DISCUSSION

The 3 groups of individuals studied being comparable, it becomes evident that on patients with occupational diseases of the lung there are significant changes on the composition of the pulmonary surfactant.

On the whole, the changes encountered on both groups of patients are similar, suggesting partially common metabolic paths. This is in accordance with other data of the study of BAL liquid referring either to cellular elements or to chemical mediators.[17,19,20,21,22,23,24]

One of the more relevant conclusions of this study is the decrease of the phospholipids that form the molecules with tensio-active function generally accepted, especially phosphatidylcholine, as it is clear on Table I.[5,3] In parallel there are alterations of many other phospholipids, whose meaning is difficult to determine since its functions have not yet been clarified.

Even more interesting are the profound modifications on the composition of fatty acids related with phospholipids knowing that, for instance, the tensio-active properties of PC depend of the fatty acids in positions α or β.[7]

It is true that for a normal surfactant function a minimal quantity of DPPC is required and that some variations in fatty acids composition do not interfere with this function.[4] On these patients it is a field requiring further research, so much for the fact that we recognize that, in exposed individuals without the disease, the composition in fatty acids did not vary from controls; suggesting that the alterations follow the surge of the disease.

One word about the great amount of arachidonic acid found in pigeon breeders without forgetting that it is on the basis of leucotrienes, Prostaglandins and Paf-acether, mediators involved in the disease.[17,22,24]

Also in relation to the composition in neutral lipids, the alterations are profound and its meaning remains uncleared, given the lack of knowledge associated with its functions.[5,18] However we would like to point out that triglycerids are the preferential form of stock of the fatty acids which, by subsequent oxidation or sterification form other lipids.[18] Also the relative decrease in fatty acids found on some patients may contribute to explain the susceptibility to acquire respiratory infections which is widely accepted.[8,9]

So we find important disturbances on the metabolic path of the surfactant synthesis. What does it mean?

Table I
Bronchoalveolar Lavage

	PIGEON %	CONTROLS %	SILICOSIS %
PHOSPHATIDYLCHOLINE	52,1±2,5 * ↓	59,6±2,9	65,8±5,4 N.S.
PHOSPHATIDYLGLYCEROL	16,3±2,2 N.S.	23,4±5,6	9,9±3,5 *** ↓
PHOSPHATIDYLINOSITOL + PHOSPHATIDYLSERINE	9,2±4,2 N.S.	9,8±2,1	8,0±2,5 N.S.
PHOSPHATIDYLETHANOLAMINE	10,4±2,7 *** ↑	4,9±1,5	7,5±1,0 ** ↑
CARDIOLIPINE	6,1±1,6 N.S.	4,9±0,9	4,8±1,6 N.S.
SPHINGOMYELINE	7,0±1,3 **** ↑	1,3±0,6	7,9±3,1 ** ↑

* $p < 0,05$ *** $p < 0,01$

** $p < 0,02$ **** $p < 0,001$

Table II
Bronchoalveolar Lavage

	PIGEON	CONTROLS	SILICOSIS
CHOLESTEROL	++++	++++	++++
FREE FATTY ACIDS	+	+	+
TRIGLYCERIDES	++	++	++
CHOLESTEROL ESTER	+++	+++	+++

We have already mentioned that in these diseases there are changes in the structure and in the microatmosphere of the alveolus that may explain its origin.[11,12] Also, we must not forget that the alveolar macrophage (AM) which, in these patients, is permanently activated producing enzymes and chemical mediators whose interaction with the Pneumocite type II is not clarified.

In parallel the cellular membrane of the activated AM is a productive source of lipidic molecules[22,24] which may contribute for the constitution of the surfactant. In the end it is possible that the Pneumocite type II may be directly injured; Schuyler[21] found in the bronchoalveolar lavage fluid of unusual silicosis patients an a burdance of Pneumocites type II so far unexplained.

In the pathogenesis of occupational diseases of the lung there are mechanisms that may cause modifications in the synthesis and secretion of the surfactant.

We believe however these changes to be more than an epiphenomenon. It is probable that they condition functional changes of the pulmonary surfactant and therefore enable a positive loop of amplification of the processes in course.

Let us not forget that in the acute phases of the disease there is interstitial edema an alveolar transudation and that in other phases alveolar collapse appears. What is the role of surfactant alterations in these processes? Can these changes disturb its basic role in the muco-ciliar stair?

CONCLUSIONS

In patients with occupational diseases of the lung there are important alterations in the lipidic composition of the pulmonary surfactant. Future research will be necessary to establish up to which point these changes disturb its functions contributing to the clinical-pathological picture of the diseases.

REFERENCES

1. PATTLE R.E.—Properties, Function and Origin of Alveolar Lining Layer. *Nature (London)*, 175:1125-1126 (1955).
2. CLEMENTS J.A.—*Proc. Soc. Exp. Bivl. Med.*, 95:170-172 (1957).
3. VAN GOLDE L.M.G.—Synthesis of Surfactant Lipids in the Adult Lung. *Am. Rev. Phvsiol.*, 47:765-774 (1985).
4. SHELLEY S.A., Paciga J.E., Balis J.U.—Lung Surfactant Phospholipids in Different Animal Species. *Lipids*, 19:857-862 (1984).
5. KING R.J.—Pulmonary Surfactant. *J. Appl. Physiol.*, 53:1-8 (1982).
6. CATENA E., Marcatili S., Marzo C., Martin A., Rogliani E.—The Components of Alveolar Surfactant of the Human Lung in Normal and in Pathological States. Pulmonary Surfactant System. *Elsevier Science Publishers*, 33-38 (1983).
7. HOLLINGSWORTH M., Gillillan M.—The Pharmacology of Lung Surfactant Secretion. *Pharmacological Reviews*, 36:69-90 (1984).
8. DOONROD J.D.—Role of Surfactant Free Fatty Acids in Anti-microbial Defenses. *Eur. J. Resp. Dis.*, 71 (Supl. 153):209-214 (1987).
9. O'NEILL S.J. Lesperance E., Klan D.J.—Human Lung Lavage Surfactant Enhances Staphylococcal Phagocytosis by Alveolar Macrophages. *Am. Rev. Resp. Dis.*, 130:1177-1179 (1984).
10. KING R.J.—Composition and Metabolism of the Apoliproteins of Pulmonary Surfactant. *Am. Rev. Physiol.*, 47:775-788 (1985).
11. FISHER A.B., Chander A.—Intracellular Processing of Surfactant Lipids in the Lung. *Am. Rev. Physiol.*, 47:789-802 (1985).
12. OYARZUM M.J., Clements J.A.—Control of Lung Surfactant by Ventilation, Adrenergic Mediators and Prostaglandins in Rabbit. *Am. Rev. Resp. Dis.*, 117:879-891 (1978).
13. FARREL P.M., Avery M.E.—Hyaline Membrane Disease. *Am. Rev. Resp. Dis.*, 111:657-88 (1975).
14. HALLMAN M., Spragg R., Hanell J.H., Moser K.M., Gluck L.—Evidence of Lung Surfactant Abnormality in Respiratory Failure. Study of Bronchoalveolar Lavage Phospholipids, Surface Activity, Phospholipasis Activity and Plasma Nyoinositol. *J. Clin. Invest.*, 70:673-683 (1982).
15. DUNNILL M.S.—Industrial Lung Diseases. Pulmonary Pathology. *Churchill Livingstone 2ed. Ed.*, 487-5529 (1987).
16. FREITAS E COSTA M., Teles de Araujo-Le Fibrosé Polmonary. *Federazione Medica*, 36:859-870 (1983).
17. DAVIS G.S., Calhoum W.J.—Occupational and Environmental Causes of Interstitial Lung Disease. Interstitial Lung Disease. *Ed. B.C. Decker Inc.*, 63-109 (1988).
18. MASON R.J.—Lipid Metabolism in the Biochemical Basis of Pulmonary Function. *Ed. Ronald G. Crystal. Marcel Decker Inc.*, 127-169 (1976).

Table III

	PIGEON	CONTROLS	SILICOSIS
12:0	1,1±0,2 N.S.	1,4±0,6 N.S.	1,9±1,1 N.S.
14:0	1,1±0,2	2,1±0,8	4,5±0,9 * ↑
14:1	< 1	< 1	1,1±0,9
16:1	5,6±2,2 ↓ ***	59,7±2,8	25,9±2,9 ↓ ***
16:1	< 1	< 1	< 1
16:2	< 1	< 1	< 1
16:3	21,8±1,5 ↑ ***	< 1	13,8±1,3 ↑ ***
18:0] 18:1]	42,9±2,9 N.S.	24,5±7,4	30,2±2,2 N.S.
18:2	< 1 ↓ **	6,1±2,9	3,2±3,3
18:3	4,3±0,9 ↑ **	< 1	13,1±0,9 ↑ **
20:0] 20:1]	< 1	< 1	7,6±1,6 ↑ **
20:4	22,6±4,1 ↑ **	8,7±2,5	4,4±3,6 N.S.

* $p < 0,05$ ** $p < 0,01$ *** $p < 0,001$

Table IV

Ratio Total Saturated Fatty Acids/Total Unsaturated Fatty Acids

PIGEON		CONTROLS		SILICOSIS
0,3 ± 0,1 ←	S***	→ 2,3 ± 0,6 ←	S***	→ 0,7 ± 0,1

S *** $p < 0,001$

Table V

	PIGEON	CONTROLS	SILICOSIS
12:0	< 1 N.S.	< 1 N.S.	4,2±0,7 N.S.
14:0	< 1 ↓ **	6,4±2,2	4,6±0,7 N.S.
14:1	3,4±2,4 N.S.	3 ± 2 N.S.	1,8±0,4 N.S.
16:0	13,2±9,0 ↓ *	44,9±19,5	23,9±1,2 ↓ **
16:1	2,1±1,9	< 1	< 1
16:2	< 1	< 1	< 1
16:3	8,0±1,44 ↑ **	< 1	13,0±1,2 ↑ **
18:0] 18:1]	11,2±9,9 N.S.	21,8±18,2 N.S.	32,4±9,2 N.S.
18:2	13,7±2,4 ↑ **	< 1	< 1
18.3	< 1	< 1	13,1±0,6 ↑ **
20:0] 20:1]	14,4±3,1	13,8±3,4	< 1 ↓ **
20:4	19,8±7,5 N.S.	11,7±7,7 N.S.	8,1±0,8 N.S.
22:1	8,6±6,8 N.S.	9,25±8,6 N.S.	3,3±1,2 N.S.

S * $p < 0,05$ S ** $p < 0,02$

Table VI

Ratio Total Saturated Fatty Acids/Total Unsaturated Fatty Acids

PIGEON		CONTROLS		SILICOSIS
0,3 ± 0,2	S ***	← 1,9 ± 0,8 →	S **	0,8 ± 0,1

S *** $p < 0,01$ S ** $p < 0,05$

19. DAVIS G.S.—Pathogenesis of Silicosis: Current Concepts and Hypothesis. *Lung*, 164:139-154 (1985).
20. BEGIN R.O., Cantin A.M., Boileau R.D., Bisson G.Y.—Spectrum of Alveolitis in Quartz—Exposed Human Subjects. *Chest*, 92:1061-1067 (1987).
21. SCHYLER M.R., Gaumer H.R., Stankus R.P., Hoffman E., Naimal J., Salvaggio J.E.—Bronchoalveolar Lavage in Silicosis. *Lung*, 157:95-102 (1980).
22. TELES DE ARAÚJO A., Reis Ferreira J.M., Freitas e Costa M., Benveniste J.—Abnormalities of Lipidic Composition of Bronchoalveolar Lavage in Respiratory Diseases Induced by Dust Inhalation. Sarcoidosis and Other Granulomatous Disorders. *Elsevier Science Publishers BV:* 685-688 (1988).
23. TELES DE ARAÚJO A., Mendes A.C., Monteiro J.T., Freitas e Costa M.—Characteristics of the Lymphocytary Alveolitis of Sarcoidosis and Mineral Dust Inhalation Induced Pulmonary Granulomatosis. Sarcoidosis and Other Granulomatous Disorders. *Elsevier Science Publishers BV:* 711-712 (1988).
24. SIMÕES M.H. Teles de Araujo A., Arnoux B., Palma Carlos A.G., Freitas e Costa M., Benveniste J.—The Release of Paf-acether and Lyso-Paf-acether Bronchoconstrictive Mediators by Alveolar Macrophages of Granulomatosis. Proceedings of the IV European Conference on Sarcoidosis and Other Granulomatous Disorders. *Ed. Blasi, Olivieri, Pezza*, pag 229-234 (1983).
25. BLIGH, E.G., Dyer, W.G.. *Can. J. Biochem. Physiol.*, 37:911-917 (1959).
26. CMELIK S., Fonseca E.. *Hoppe-Seyler's Z. Physiol. Chem.*, 355:19-26 (1974).
27. METCALFE, L.D., Schmitz A.A.. *Anal. Chem.*, 33:363-364 (1961).
28. LIBERMANN-BURCHARD, modif. by Stadtman T.C.—Methods in Enzymology—Vol. 3 (Colowick, S.P., Kajlan, N.O. eds). *Academic Press, Inc., New York*, pp. 393-394 (1957).
29. FISKE C.H., Subbarow J.. *J. Biol. Chem.*, 66:375-400 (1925).
30. KING E.J.. *Biochem. J.*, 26:292-297 (1932).
31. LOWRY O.H., Rosebrough N.H., Farr A.L., Randall R.J., *J. Biol. Chem.*, 193:265-275 (1951).

THE TREATMENT OF OBSTRUCTIVE AIRWAY DISEASE OF COAL WORKERS WITH COAL WORKERS' PNEUMOCONIOSIS

W. T. ULMER • I. Zimmermann • D. Schött • H. Straub

Pneumoconiosis Research Institute, Bochum/FRG

ABSTRACT

In categories B and C (ILO-classification 1980) coal workers develop more than twice as often obstructive airway diseases as persons of the same age who are not exposed to dust. The obstructive airway disease of coal workers responds in the same way on the same kind of treatment as the obstructive airway disease of non-exposed persons. Under a well-controlled treatment, the obstructive airway disease of coal miners, the most important complication of coal workers' pneumoconiosis, can be controlled, and life expectancy of these coal miners with coal workers' pneumoconiosis and obstructive airway disease is the same as that on non-exposed persons.

No Paper provided.

NUMBER, NATURE AND SIZE OF ASBESTOS BODIES IN BAL FLUIDS OF CHRYSOTILE WORKERS

P. DUMORTIER • P. De Vuyst • P. Strauss* • J. C. Yernault

Chest Department, Erasme Hospital and
*Occupational Disease Fund, Brussels, Belgium

ABSTRACT

Bronchoalveolar lavage (BAL) fluids from 15 workers of the same brake lining factory (Group 1) were investigated with respect to their asbestos bodies (AB) content. Those subjects were only exposed to chrysotile. BAL fluids from 34 asbestos cement workeres (Group 2) were also examined for comparison. Group 2 was mainly exposed to amphiboles. AB concentration was ranging from 0.2 to 3168 AB/ml (mean: 263 ± 812) for group 1 and from 0.3 to 11,200 AB/ml (mean: 1028 ± 2326) for group 2. Repeated BAL were obtained for 3 subjects of group 1. There were no significant changes in AB concentrations even 10 months after cessation of exposure. Among 159 typical AB cores analyzed in 7 subjects of group 1, chrysotile was identified in 95.6%, amosite in 2.5% and 1.9% remained undetermined. Neither tremolite-actinolite nor anthophyllite were identified as AB cores in this group. This contrasts with the data obtained on 561 AB cores from 20 subjects of group 2 where 9.8% were built on chrysotile. Geometric mean length and diameter were respectively shorter and thinner for chrysotile AB cores than for amphibole ones but mean aspect ratio was higher.

We can conclude that 1) routine AB counting in BAL samples allows to disclose occupational exposures to chrysotile, 2) such exposures can lead to AB concentration in BAL comparable to those encountered with occupational exposures to amphiboles, 3) size characteristics of AB on chrysotile are different from those of AB on amphiboles and 4) mechanisms of chrysotile fibers clearance does not substantially affect chrysotile AB concentrations in BAL for at least 10 months after cessation of exposure.

No Paper provided.

ASBESTOS BODIES IN BRONCHOALVEOLAR LAVAGE FLUID IN VIEW OF OCCUPATION, PLEURAL CHANGES, AND BRONCHOGENIC CARCINOMA

LUDOVIC M. LACQUET, M.D., Ph.D
Universitaire Ziekenhuizen, Leuven, Belgium

The asbestos content of lung tissue reflects the asbestos exposure during life. One finds both naked or uncoated fibres, and fibres coated with a ferroproteinous material also called ferruginous bodies or asbestos bodies (AB's).[1,5] The great majority of the AB's contain an amphibole in its core.[2] AB's can easily be identified in bronchoalveolar lavage fluid.[4,6] Their number correlates with the number found in lung tissue.[3] The aim of the present study is to explore the relationship between AB concentration in bronchoalveolar lavage fluid, on the one hand, and the occupational history, the presence of benign pleural changes, and the presence of primary bronchogenic carcinoma, on the other hand.

MATERIAL AND METHODS

Bronchoalveolar lavage was performed during routine diagnostic bronchoscopy, under local anesthesia, in 275 consecutive patients (257 male, mean age 60.9 ± 9.2 yr) who could tolerate the procedure safely. A fiberoptic bronchoscope was securely wedged in a segment of either the middle lobe or the lingula, which was then washed with a minimum of two aliquots of 50 ml of normal saline solution. About 50% of the second aliquot was recovered and used for analysis.

About 20 ml of lavage fluid, mixed with 40 ml of bleach, was incubated at 40°C for 2 hours. The fluid was then sucked through a 0.45 micrometer pore size cellulose esters filter, subsequently rinsed twice with water and once with alcohol 25%. The filters, mounted and cleared, were examined at 400 × magnification under phase contrast. A known portion of the filter, approximately 400 fields, were examined for AB's, from which the original concentration of AB's could be calculated. The logarithmic means were used in all comparisons; one AB was added to all counts so that no concentration came out lower than 0.01/ml, which is an artifact.

Complete occupational and smoke histories were taken. The chest radiographs were read according to the ILO classification system for pneumoconioses. The following benign pleural changes were considered for analysis: pleural effusion, diffuse pleural thickening, markedly obliterated costophrenic angle, pleural plaques calcified or not.

Control groups consisted of an equal number of patients, matched for sex, age (± 4 yr), and smoking habit (± 50% of total cigarettes smoked). Asbestos workers, i.e. workers with explicit asbestos exposure, were eliminated from matched comparisons for fear of bias. Indeed most of these were referred explicitly for detection of AB's.

RESULTS

The concentrations of AB's in lavage fluid, range 0.01–130/ml, appear to follow a logarithmic distribution in this sample of 275 patients. The 257 male subjects could be categorized in five groups, unmatched for age and smoking history, according to occupation. These groups and corresponding mean AB concentrations are: (a) 11 asbestos workers, 21.9 ± 41/ml; (b) 50 metal workers, welders, plumbers, and heating workers, 1.4 ± 7.8/ml; (c) 47 coal miners, 1.4 ± 7.9/ml; (d) 103 other blue collar workers, 0.3 ± 5.4/ml; and (e) 46 farmers, staff, and other white collar workers, 0.1 ± 5.7/ml. The mean concentrations found in the first four groups are all significantly higher than in the one found in the last group.

Metal workers (n=25) also had significantly higher AB counts if compared, not with group (e), but with an equal number of matched controls (1.23/ml versus 0.34/ml, P=0.02). The same was found for coal miners (n=37, 1.78/ml versus 0.23/ml, $P=10^{-5}$).

Likewise, subjects with bilaterial pleural changes (n=50) had significantly higher AB counts than their matched controls (1.8/ml versus 0.3/ml, 2P=0.001). However, subjects with unilateral, pleural changes (n=31) did not differ significantly from the controls (0.7/ml versus 0.4/ml).

The patients with primary bronchogenic carcinoma (n=69) had a higher mean AB concentration than matched controls, but the difference was not significant (0.76/ml versus 0.44/ml, P=0.1).

DISCUSSION

It is not surprising that the highest AB concentrations were found in asbestos workers. Of more interest is the increased number of AB's found in people who, in the majority of cases, did not mention any contact with asbestos. They constitute nevertheless a group of workers with probable asbestos exposure: steel and foundry workers, metal construction workers, welders, plumbers, central heating workers, and other workers who may use asbestos as sealing or heat insulator. It is somewhat surprising that a similar distribution of AB concentrations was found in coal miners. The Belgian geological structures contain no asbestos, but the material has been applied in the past as fire protection, among other uses. Another possible explanation is that some of these AB's

have cores made of carbon fibre rather than asbestos. The present study cannot answer this.

We have distinguished a group of blue collar workers, who definitely had no explicit occupational exposure to asbestos, although sporadic contact remains possible. This group too had a significant, but small, increase of AB's, in comparison with the group of people with occupations outside of industry. These non-industrial workers have rather low AB concentrations: less than 1/ml in 67% of them, less than 3/ml in 84%, and less than 10/ml in 93%. Even this group shows some overlap with all other groups. We do not know for sure the source of occasional high AB counts, but in some cases the hobby activity may have been responsible.

It is known that asbestos exposure can cause any of the benign pleural changes we have considered in this study. We have treated these entities as a whole. Separating them is often difficult, as often they occur together, radiologically they may overlap, and finally one type of lesion can evolve into another (e.g. pleural effusion into pleural thickening). This study shows clearly, perhaps not surprisingly, that bilateral benign pleural changes (of any type are much more indicative of previous asbestos exposure than are the unilateral ones.

The relationship of substantial asbestos exposure and increased incidence of bronchogenic carcinoma is well established. We tested the more subtle hypothesis, that bronchogenic carcinoma in the general population might develop more frequently in the presence of a moderate level of asbestos impregnation of the lungs. To elucidate this, the group of 69 male patients with bronchogenic carcinoma was compared with a group of matched controls. The carcinoma cases indeed showed a higher mean AB concentration, but the difference was not significant although one might suspect a trend ($P=0.1$). This is in agreement with studies wherein the asbestos contents of lung tissue were compared.[7] A major influence thus of moderate asbestos impregnation on the incidence of lung cancer does not seem to exist. The final answer to this question must await the comparison of larger groups and identification of the cores of the AB's also would be desirable.

CONCLUSIONS

Our study of AB's in bronchoalveolar lavage fluid yields the following conclusions: (a) There is a gradation of AB concentrations related to occupational history; (b) increased AB concentrations correlate with the presence of benign pleural changes, when visible bilaterally on a standard chest radiograph; and (c) AB concentrations are not significantly increased in men, non-asbestos workers, with bronchogenic carcinoma.

REFERENCES

1. Churg, A.: Fiber Counting and Analysis in the Diagnosis of Asbestos Related Disease. *Hum. Pathol.* 13:381-392 (1982).
2. Churg, A., Warnock, M.L., Green, N.: Analysis of the Cores of Ferruginous (Asbestos) Bodies from the General Population. II. True Asbestos Bodies and Pseudoasbestos Bodies. *Lab Invest.* 40:31-38 (1979).
3. De Vuyst, P., Dumortier, P., Moulin, E., Yourassowsky, N., Roomans, P., de Francquen, P., Yernault, J.C.: Asbestos Bodies in Bronchoalveolar Lavage Reflect Lung Asbestos Body Concentration. *Eur. Respir. J.* 1:362-367 (1988).
4. De Vuyst, P., Jedwab, J., Dumortier, P., Vandermoten, G., Van de Weyer, R., Yernault, J.C.: Asbestos Bodies in Bronchoalveolar Lavage. *Am. Rev. Respir. Dis.* 126:972-976 (1982).
5. Morgan, A., Holmes, A.: Concentration and Dimension of Coated and Uncoated Fibers in Human Lungs. *Br. J. Ind. Med.* 37:25-32 (1980).
6. Sebastien, P.: Possibilites Actuelles de la Biometrologie des Poussieres sur Echantillons de Liquide de Lavage Bronchoalveolaire. *Ann. Biol. Clin.* 40:279-293 (1982).
7. Whitwell, F., Scott, J., Grimshaw, M.: Relationship Between Occupations and Asbestos-Fibre Content of the Lungs in Patients with Pleural Mesothelioma, Lung Carcinoma and Other Diseases. *Thorax* 32:377-386 (1977).