

**TYPE I AND III PROCOLLAGEN
PROPEPTIDES IN
SARCOIDOSIS, FIBROSING
ALVEOLITIS AND ASBESTOS-
RELATED LUNG DISEASES**

**LAURI
LAMMI**

Department of Internal Medicine

OULU 1999



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DISEASES**

Academic Dissertation to be presented with the assent of the Faculty of Medicine, University of Oulu, for public discussion in Auditorium 10 of the University Hospital of Oulu, on October 22nd, 1999, at 12 noon.

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To my family

Lammi Lauri, Type I and III procollagen propeptides in sarcoidosis, fibrosing alveolitis and asbestos-related lung diseases

Vaasa Central Hospital, Department of Pulmonary Medicine, FIN 65130 Vaasa and Department of Internal Medicine, University of Oulu, P.O. Box 5000, FIN 90401 Oulu 1999

Oulu, Finland

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Abstract

The most threatening outcome of interstitial lung diseases is death caused by progressive pulmonary fibrosis characterised by increased collagen deposition, although the clinical course is highly variable. The aim of this study was to evaluate the role of procollagen I and III propeptides in estimating collagen metabolism and its relationship to disease activity and prognosis in patients with sarcoidosis, fibrosing alveolitis and asbestos-related lung diseases.

The study included 160 patients. The levels of procollagen I carboxyterminal propeptide (PICP) and procollagen III aminoterminal propeptide (PIIINP) in serum, bronchoalveolar lavage fluid (BALF) and epithelial lining fluid (ELF) were assessed from 137 patients employing human antigens. There were 60 patients with sarcoidosis, 18 with fibrosing alveolitis and 5 with asbestosis and 17 controls. Thirty-seven patients had been exposed to asbestos, but did not show parenchymal involvement. Twenty-five of them had pleural plaques, while 12 had normal chest radiographs. Immunohistochemical stainings for procollagen I aminoterminal (PINP) and III aminoterminal propeptide were carried out on open lung biopsies of the remaining 23 of the 160 patients, of whom 13 had sarcoidosis and 10 fibrosing alveolitis. Antibodies to these procollagen peptides react with the aminoterminal domains of the corresponding propeptides intracellularly and with the respective pN-collagen in collagen fibres in the extracellular space.

Procollagen III aminoterminal propeptide was elevated in the sera of the patients with sarcoidosis and fibrosing alveolitis, but not in the asbestosis or asbestos-exposed patients as compared to the controls. The level of PIIINP in BALF was highest in sarcoidosis and second highest in fibrosing alveolitis, but hardly detectable in the other groups. BALF-PICP was higher in the patients with fibrosing alveolitis, sarcoidosis and asbestosis than in the controls. PIIINP in BALF correlated with BALF-PICP, serum angiotensin-converting enzyme (S-ACE), interleukin 2-receptor, BALF-albumin and BALF-lymphocytes and BALF-PICP had a significant correlation with BALF-albumin and BALF-lymphocytes in sarcoidosis. BALF/ELF-PICP had an inverse correlation with the specific diffusion coefficient (DLCO/VA) in fibrosing alveolitis. Both PIIINP and PICP were higher in ELF than in serum in sarcoidosis and fibrosing alveolitis and PICP was higher in ELF compared to serum in asbestosis, suggesting active local synthesis in the lower respiratory tract. The levels of PIIINP in BALF were significantly elevated in sarcoidosis patients with parenchymal involvement compared to those without. Detectable PIIINP in BALF also predicted a poor outcome in fibrosing alveolitis. BALF-PIIINP reflected the disease activity based on chest radiographs in sarcoidosis and a poor prognosis in fibrosing alveolitis, whereas BALF-PICP marked the development of fibrosis.

In lung biopsy specimens, type I and III pN-collagens were increased in fibrosing alveolitis and sarcoidosis. Type I pN-collagen was expressed in areas with damaged or deficient alveolar epithelium. Type III pN-collagen was present underneath regenerative, metaplastic alveolar and bronchiolar type epithelium and was accumulated both in the loose, newly formed fibrosis and in the denser old fibrosis. Type I procollagen was present in intracellular spots in newly formed fibrosis. In sarcoidosis, type I procollagen was present intracellularly in granulomas, whereas type III pN-collagen was expressed extracellularly around granulomas.

Keywords: Collagen metabolism, collagen markers, bronchoalveolar lavage fluid

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Vaasa, August 1999

Lauri Lammi

Abbreviations

AB	asbestos body
ACE	angiotensin-converting enzyme
AIP	acute interstitial pneumonia
ARDS	adult respiratory distress syndrome
BAL	bronchoalveolar lavage
BALF	bronchoalveolar lavage fluid
BM	basement membrane
CT	computed tomography
DIP	desquamative interstitial pneumonia
DLCO	diffusion capacity
DLCO/VA	specific diffusion coefficient
ELF	epithelial lining fluid
FEV1	forced expiratory volume in one second
FVC	forced vital capacity
HRCT	high-resolution computed tomography
IL-2R	interleukin 2-receptor
NSIP	non-specific interstitial pneumonia
PICP	procollagen I carboxyterminal propeptide
PINP	procollagen I aminoterminal propeptide
PIIINP	procollagen III aminoterminal propeptide
pN-collagen	collagen molecule with retained aminoterminal propeptide
UIP	usual interstitial pneumonia

List of original communications

This thesis is based on the following articles, which are cited in the text using the Roman numerals I-IV:

- I Lammi L, Kinnula V, Lähde S, Risteli J, Pääkkö P, Lakari E & Ryhänen L (1997) Propeptide levels of type III and type I procollagen in the serum and bronchoalveolar lavage fluid of patients with pulmonary sarcoidosis. *Eur Respir J* 10: 2725-2730.
- II Lammi L, Ryhänen L, Lakari E, Risteli J, Pääkkö P, Kahlos K, Lähde S & Kinnula V (1999) Type III and type I procollagen markers in fibrosing alveolitis. *Am J Respir Crit Care Med* 159:818-823.
- III Lammi L, Ryhänen L, Lakari E, Risteli J, Pääkkö P, Ruotsalainen EM, Lähde S & Kinnula VL (1999) Carboxyterminal propeptide of type I procollagen is elevated in the epithelial lining fluid in asbestosis, but not in asbestos related pleural plaque disease. *Eur Respir J* (in press).
- IV Kaarteenaho-Wiik R, Lammi L, Lakari E, Kinnula V, Risteli J, Ryhänen L & Pääkkö P (1999) Type I and III procollagens and pN-collagens are differentially distributed in usual interstitial pneumonia and sarcoidosis. (submitted for publication).

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1. Introduction

Interstitial lung diseases comprise a heterogeneous group of disorders of the lower respiratory tract. This clinical entity includes numerous diseases, such as sarcoidosis, fibrosing alveolitis, hypersensitivity pneumonitis of various causes, pulmonary involvement related to autoimmune diseases, collagen vascular diseases, asbestosis and other pneumoconiosis. They are characterised by both acute and chronic inflammation and a generally irreversible process of fibrosis within the interstitium and the alveolar spaces. A typical feature of interstitial lung disease is intra-alveolar and interstitial accumulation of inflammatory cells, principally neutrophils and macrophages and, less frequently, immunologically active cells. The alveolar epithelium becomes damaged and denuded, progressing towards damage of the basement membrane (BM) in association with the activation of inflammatory and immune effector cells. Matrix proteins, such as hyaluronan, are released in a soluble form from the interstitium and accumulate in the alveolar spaces. These events lead to collapse and fusion of the alveolar units, and to a fibrotic response that results in increased collagen synthesis in interstitial and intra-alveolar fibroblasts. This finally causes a loss of function in the gas exchanging units and, ultimately, respiratory failure. (Chan *et al.* 1998).

Pulmonary fibrosis starts with the accumulation of type III collagen, which is later largely replaced by type I collagen and the formation of tight collagen fibres (Bateman *et al.* 1981, Kirk *et al.* 1984). Previous measurements of collagen metabolism have indicated that procollagen III aminoterminal propeptide (PIIINP) reflects the synthesis of type III collagen, although it can, to some extent, indicate degradation of collagen III fibres. Procollagen I carboxyterminal propeptide (PICP) reflects the synthesis but not the degradation of type I collagen fibres. (Risteli & Risteli 1995). Thus, measurement of procollagen propeptides as well as other markers of collagen metabolism (e.g. unique enzymes of collagen metabolism, such as prolyl hydroxylase and galactosyl hydroxylysyl glucosyltransferase) would offer theoretically methods for evaluating collagen metabolism and the development of fibrosis. Previous studies on procollagen III aminoterminal propeptide in fibrosing alveolitis and sarcoidosis have yielded divergent results. All but one of them have been carried out with antibodies against bovine PIIINP, which has been shown to be less specific to PIIINP than human antibodies with intra- and interassay variation of 10-25% (Risteli & Risteli 1990).

Fiberoptic bronchoscopy and bronchoalveolar lavage (BAL) offer an easy and non-invasive method to obtain cells and fluid from lung parenchyma (Davis 1994). Cell-differential counting in bronchoalveolar lavage fluid (BALF) may also help in the differential diagnosis for instance neutrophilia in BALF suggests fibrosing alveolitis, whereas an excessive amount of lymphocytes suggests sarcoidosis or allergic alveolitis (Taskinen *et al.* 1994).

Sarcoidosis causes granulomatous inflammation in various organs, although the lung is the organ most frequently affected (Stirling *et al.* 1998). The prognosis is good in most cases, and most patients recover completely even without any treatment. However, 10-20% of the sarcoidosis patients are at risk for developing progressive pulmonary fibrosis (Selroos 1969, Crystal *et al.* 1984). Several markers of disease activity have been suggested, but the results have been inconclusive. The only routine tests to stage the activity of sarcoidosis have been suggested to be clinical investigation, chest radiography and lung function testing. (Costabel *et al.* 1994). Fibrosing alveolitis can be idiopathic (King 1998) or associated with various connective tissue diseases (de Andrade & Kennedy 1999, Kelly 1999). The presentation of idiopathic fibrosing alveolitis is insidious in most patients, involving a progressive course of several years, but the onset can also be acute and fulminant. A favourable response to medication, most commonly corticosteroids or cytotoxic therapy, is only seen in one third of the patients. (Katzenstein & Myers 1998). There is no single reliable prognostic factor indicative of the activity of fibrosing alveolitis. The follow-up of these patients is based on spirometry and diffusion capacity values as well as changes in chest radiography and occasionally high-resolution computed tomography (HRCT) of the lungs (Johnston *et al.* 1999). Exposure to asbestos fibres may lead to hyaline plaques of parietal pleura, asbestos pleurisy, asbestosis and malignant diseases of the lower respiratory tract and the pleura. Pleural plaques do not cause impairment of respiratory capacity, whereas asbestosis leads to slowly progressive pulmonary fibrosis caused by asbestos fibres with a latency of 15-20 years from the first exposure to asbestos (Becklake 1987). It is not known, which individuals in an exposed population tend to develop fibrosis.

Interstitial lung diseases that lead to pulmonary fibrosis cause permanent impairment of pulmonary capacity, respiratory failure and ultimately death. So far, no reliable methods to predict the course of these diseases are available. The role of procollagen III propeptide has been widely studied, but the results have been conflicting, although there is some evidence that elevated levels of procollagen III aminoterminal propeptide in BALF and serum might correlate with active disease. The information available on procollagen I propeptides is limited.

The present study was undertaken to investigate the procollagen propeptide markers of type I and III collagen in sarcoidosis, fibrosing alveolitis and asbestos-related diseases and to assess their role in predicting the prognosis of these diseases.

2. Review of the literature

2.1. Sarcoidosis

Sarcoidosis is a granulomatous inflammation of unknown origin involving various organs, most often the lungs and lymph nodes (Stirling *et al.* 1998). The prevalence of sarcoidosis is highly variable, ranging from 0.2/100000 in South America to 64/100000 in Sweden (Bauer & Löfgren 1964). The crude prevalence of sarcoidosis is 28.2 per 100000 and its annual incidence 11.4 per 100000 with a female predominance in Finland (Pietinalho *et al.* 1995). Sarcoidosis can present in an acute or subacute form, being mostly self-limiting, and the majority of patients recover spontaneously (Stirling *et al.* 1998). However, 10-20% of sarcoidosis patients are at risk for developing progressive pulmonary fibrosis as a severe and irreversible complication, which may lead to permanent disability of the patient (Selroos 1969, Crystal *et al.* 1984). These patients require prolonged treatment with corticosteroids and occasionally cytotoxic therapy. Several markers of disease activity have been suggested. The serum level of angiotensin-converting enzyme (S-ACE) is a widely used indicator of sarcoidosis activity (Sharma 1986), although it does not reflect the activity of fibrogenesis (Harf *et al.* 1988). Serum interleukin-2 receptor (S-IL-2R) has also been shown to be elevated in sarcoidosis as well as in other lung diseases (Lawrence *et al.* 1988, Tsutsumi *et al.* 1994). Other suggested markers include ⁶⁷Ga-scan, computed tomography (CT) and HRCT as well as BALF cell subpopulations. An elevated T-helper/T-suppressor (CD4/CD8)-lymphocyte ratio in BALF has been suggested to be typical of sarcoidosis, although it has been recently shown to have low sensitivity for sarcoidosis (Kantrow *et al.* 1998). The results on these various markers have been inconclusive. The World Association for Sarcoidosis and Other Granulomatous Disorders (WASOG) meeting report concluded that the only routine tests to stage the activity of sarcoidosis are clinical investigation, chest radiography and lung function testing (Coscabel *et al.* 1994).

2.2. Fibrosing alveolitis

Fibrosing alveolitis can be idiopathic (King 1998) or associated with various connective tissue diseases, e.g. rheumatoid arthritis (Kelly 1999) and systemic lupus erythematosus (de Andrade & Kennedy 1999). Epidemiological data on idiopathic fibrosing alveolitis are sparse. The prevalence range has been reported to be 6–14.6 per 100000 (Scott *et al.* 1990, Coultas *et al.* 1994). The etiology of idiopathic pulmonary fibrosis or cryptogenic fibrosing alveolitis is unknown, but there is evidence to suggest that occupational and environmental factors as well as viral, immunologic, and genetic factors are involved in the development of idiopathic fibrosing alveolitis (King 1998). The clinical features are variable. The condition is seen most often in middle-aged or older adults. The presentation is insidious in most patients with a progressive course of several years, but the onset can also be acute and fulminant. A favourable response to medication, most commonly corticosteroids or cytotoxic therapy, is shown by only in one third of the patients. (Katzenstein & Myers 1998). Idiopathic fibrosing alveolitis was recently classified pathologically by Katzenstein and Myers as follows: 1) Usual interstitial pneumonia (UIP), 2) Desquamative interstitial pneumonia (DIP)/respiratory bronchiolitis interstitial lung disease, 3) Acute interstitial pneumonia (AIP, Hamman-Rich disease) and 4) Non-specific interstitial pneumonia (NSIP) (Katzenstein & Myers 1998). UIP is the most common type of idiopathic fibrosing alveolitis, accounting for over 60% of the cases (Bjoraker *et al.* 1998). The course of UIP is chronic and the prognosis is poor, with mortality ranging from 59% to 70%. Patients with DIP are usually younger than patients with UIP, and their prognosis is better with mortality of 5% in five years. There is a clear predominance of men in UIP and DIP. The prognosis of AIP is worst with a mortality range of 50% to 80% and most deaths occurring within 1 to 2 months. Mortality from NSIP is 15-20% in five years. To distinguish between these entities, it is recommendable to perform open lung biopsy. (Katzenstein & Myers 1998, King 1998). Currently, there is no single reliable prognostic factor for the activity of cryptogenic fibrosing alveolitis. The follow-up is mostly based on spirometry and diffusion capacity values, chest radiography and occasionally high-resolution computed tomography (HRCT) of the lungs (Johnston *et al.* 1999).

2.3. Asbestos-related lung diseases

Occupational or other environmental exposure to asbestos fibres may lead to hyaline plaques of parietal pleura, asbestos pleurisy, and asbestosis. Asbestos-induced malignancies of the respiratory tract include bronchogenic carcinoma and mesothelioma of the pleura. (Schwartz & Peterson 1998). Asbestosis leads to slowly progressive pulmonary fibrosis with a poor prognosis and a latency of 15-20 years from the first exposure to asbestos (Becklake 1976). The duration and intensity of asbestos exposure correlate to the quantity of fibres remaining in lung tissue and the quantity of asbestos bodies (AB) in bronchoalveolar lavage fluid (Davis 1994). There is a clear dose-response relationship between the severity of exposure of asbestos and the development of asbestosis. Pleural plaques and diffuse pleural thickening are also dependent on the cumulative dose of

inhaled asbestos. 20-60% of construction workers exposed to high concentrations of asbestos develop signs of pleural fibrosis in chest radiographs. (Koskinen *et al.* 1998, Schwarz & Peterson 1998). It is unclear which individuals in an asbestos-exposed population are at risk of progressive pulmonary fibrosis. The chest radiographic finding is abnormal in a minority of the cases of pathologically demonstrated asbestosis (Kipen *et al.* 1987), and HRCT of the lungs is recommended for a diagnosis of asbestosis, especially in the early stages (Lynch 1998).

2.4. Procollagen and collagen synthesis

Collagens are the major fibrillar components of most connective tissues. At least nineteen different subtypes of collagens have been identified (Prockop & Kivirikko 1995). The collagens I, III, IV, V and VI are known to predominate in normal and fibrotic lungs (Madri & Furthmayr 1980, Raghu *et al.* 1985, Specks *et al.* 1995). Collagens form a family of proteins, and each type has its own characteristic amino acid sequence. Each collagen molecule consists of three individual polypeptides known as α -chains. Each α -chain has a left-handed helical secondary structure around its axis. Three α -chains are then coiled around each other into a right-handed superhelix. This triple-helical conformation is unique to collagen. (Uitto *et al.* 1981).

Interstitial collagens are synthesised and secreted out of cells as procollagens. During the secretion, carboxy- and aminoterminal propeptides are cleaved off from the parent molecule by specific proteases (Prockop *et al.* 1979). This releases the collagen molecule, together with its respective procollagen propeptides, in stoichiometric amounts into the extracellular space (Fig. 1). The propeptide molecules are then further degraded by non-specific proteases to smaller peptides, which retain the antigenicity of the parent procollagen propeptide molecule (Rohde *et al.* 1983). The function of the carboxyterminal propeptide is to direct the assembly of the three polypeptide chains and to initiate their winding into a triple-helical conformation. The cleavage of carboxyterminal propeptide seems to be a prerequisite for the assembly of collagen fibres. (Miyahara *et al.* 1982). The cleavage of the aminoterminal propeptide can be incomplete, resulting in the formation of a collagen molecule with attached aminoterminal propeptide (pN-collagen). This is typical of type III collagen, and such molecules have been observed *in vivo* on the surface of type III collagen fibrils (Karttunen *et al.* 1989). It is known that when these fibrils are degraded, type III pN-collagen is released into the extracellular space (Risteli & Risteli 1986). Antigenicity related to PIIINP is found in human serum with different molecular sizes. The largest form may represent intact type III pN-collagen or procollagen, the second form is larger than the authentic propeptide, the third form is similar in size to the authentic propeptide, and the smallest form is obviously derived from further degradation of the others and it predominates in human serum (Niemelä 1985, Niemelä *et al.* 1985). The commercial radioimmunoassays employing bovine antigen also detect the procollagen or pN-collagen antigenicity, whereas the assay based on human antigen detects the second and third forms as described above, being more sensitive to the synthesis of type III collagen. (Niemelä 1985, Risteli & Risteli 1990). Cleaved procollagen propeptides disappear during the processing of histological specimens. The intracellular immuno-

reactivity of aminoterminal propeptides detects the intracellular aminoterminal propeptide domain of the corresponding procollagen, and extracellularly they react with the amino-terminal domain still attached to the corresponding pN-collagen molecule.

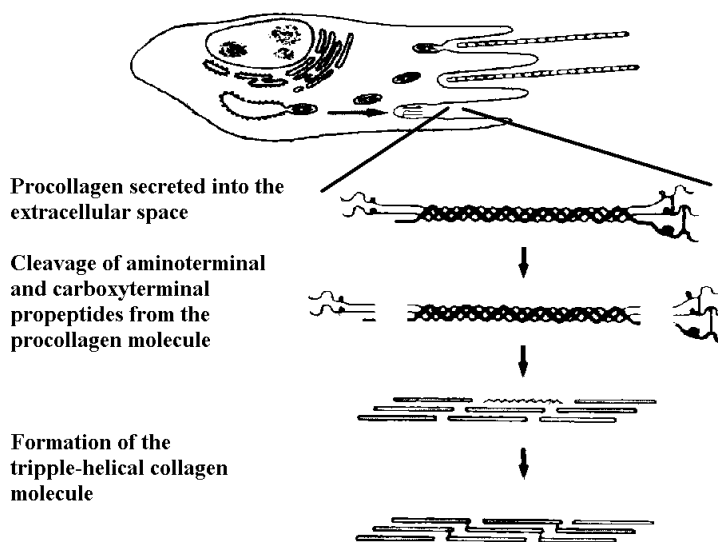


Fig. 1. Schematic presentation of the extracellular processes of collagen synthesis. Modified from Prockop & Kivirikko (1984).

2.5. Use of collagen markers in diseases

Collagen propeptides have been widely investigated in different physiological stages and various fibrosing conditions other than pulmonary fibrosis (Risteli & Risteli 1990). In wound healing, PIIINP is elevated in serum (Bentsen *et al.* 1988) and *in situ* in the interstitial fluid of a healing wound (Haukipuro *et al.* 1987). The serum concentrations of PICP (Carey *et al.* 1985) and PIIINP (Risteli *et al.* 1988) are significantly elevated in growing infants and children compared to adults. The normal values of serum PICP vary in males and females within $40\text{-}200\mu\text{gL}^{-1}$ and $50\text{-}170\mu\text{gL}^{-1}$, respectively. Serum PIIINP increases during the last trimester of pregnancy (Risteli *et al.* 1987).

Serum PIIINP has been widely studied in several hepatic disorders and is elevated in various pathological conditions of the liver (Risteli & Risteli 1990). For instance, serum PIIINP is increased in primary biliary cirrhosis, showing an association with the disease prognosis (Niemelä *et al.* 1988). Both S-PIIINP and S-PICP are elevated in alcoholic liver disease, and S-PIIINP correlates with the severity of the disease (Niemelä *et al.* 1990, Niemelä *et al.* 1992). Serum PIIINP is also elevated during the development of fibrosis in other organs, most markedly in bone marrow fibrosis (Hasselbach *et al.* 1990). The level of PIIINP in serum is increased in various malignancies, correlating with their clinical extent and behaviour (Zhu *et al.* 1994).

2.6. Bronchoalveolar lavage

Bronchoalveolar lavage is a diagnostic procedure of washing a sample of cells and secretions from the alveolar and bronchial airspaces. It is performed by installing commercial sterile 0.9% saline solution for intravenous use via a channel of a fiberoptic bronchoscope, which has been wedged into a bronchus with a matching diameter. The fluid is then immediately withdrawn. The instilled fluid fills the airspaces distal to the tip of bronchoscope, replacing the air. A portion of the installed volume remains to be absorbed or expectorated after the procedure. Saline solution is usually used at room temperature, and warming it up to 37°C does not involve any advantage. (Davis 1994). After withdrawal, the fluid should be kept on ice until it is further processed.

A major clinical limitation for the utility of bronchoalveolar lavage is the large range of normal values for each parameter, which makes BALF insensitive in detecting disease. Furthermore, abnormalities in BALF are rarely specific for any of the interstitial lung diseases. There are some subjects who have normal BALF constituents despite a definite disease and some without any evidence of disease despite abnormal BALF findings. There is large interindividual variation which may not be related to the disease, and the airspace cells and secretions may not reflect interstitial processes. The removal of BALF may preferentially select, activate or injure some cells, and the composition of the epithelial lining fluid (ELF) may change during the bronchoalveolar lavage. (Davis 1994).

2.6.1. Cell findings in bronchoalveolar lavage fluid

The standard techniques for investigating the cells in bronchoalveolar lavage fluid include millipore filter preparations stained with Papanicolaou and cytocentrifuge preparations stained with May-Grünwald-Giemsa. A routine cytological examination of BALF includes the determination of total and differential cell counts, screening for foreign particles and microbes, and recognition and classification of cellular atypia. In the differential count used in routine diagnostic purposes, 200 consecutive cells are counted and expressed as percentages of the whole cell population. The proportion of red blood cells is used to evaluate blood contamination in BALF. (Taskinen *et al.* 1994).

The cell findings in BALF are non-specific in most of cases. An increased total amount of cells ($>150 \times 10^6/l$ among non-smokers and $>360 \times 10^6/l$ among smokers) in BALF is common and can be seen with most diseases. Smoking causes a three- to four-fold increase in the total cell count, mainly because of increased macrophages. The highest concentrations ($>800 \times 10^6/l$) can be seen in acute bacterial infections, eosinophilic pneumonias and leukaemias and, a considerable increase ($>400 \times 10^6/l$) may appear in allergic alveolitis and infections. In sarcoidosis, the total amount of cells is typically only slightly elevated. A normal total count of cells in BALF can be seen in fibrosing alveolitis with a prolonged course, various fibrosing processes, cytomegalovirus infections in immunocompromised patients and treated or low-activity sarcoidosis. (Taskinen *et al.* 1994).

The pattern and numbers of cells recovered in fibrosing alveolitis are significantly abnormal. The absolute numbers of macrophages, neutrophils, eosinophils and basophils are usually increased, and lymphocytes are variably increased as well. The percentages of neutrophils, eosinophils, basophils and sometimes lymphocytes are increased, thus diminishing the percentage of macrophages. An elevated fraction of lymphocytes has been associated with a good prognosis and response to therapy, while elevated eosinophils are related to a poor prognosis. (Davis 1994).

There are no BALF abnormalities that would be specific to sarcoidosis or alone diagnostic of this disease. A high percentage of lymphocytes (>30%) with predominating CD4 T-cells strongly suggests sarcoidosis whenever the clinical picture is compatible to sarcoidosis. Hypersensitivity pneumonitis involves typically lymphocytic alveolitis with a predominance of CD8 T-cells. (Davis 1994). The recent results have suggested that the CD4/CD8 T-lymphocyte ratio in the BALF of sarcoidosis patients is variable (Kantrow *et al.* 1997).

2.6.2. Soluble constituents in bronchoalveolar lavage fluid

The proteins of serum constitute at least two thirds of the proteins in BAL fluid. Some of them are synthesised only outside the lungs, such as albumin, while some are produced both within and outside the lungs, such as immunoglobulins, and a few are produced only in the respiratory tract, such as surfactant. Many pulmonary diseases result in an increase of total proteins and other constituents derived from serum, which probably reflects the increased permeability associated with inflammation. In fibrosing alveolitis, total proteins, immunoglobulins and sometimes albumin are elevated in BALF. The increase of proteins of serum origin with high molecular weight suggests altered permeability. (Davis 1994).

Urea can be measured in BALF and can be used to estimate the amount of epithelial lining fluid (ELF). The original concentration of urea is the same in serum and in ELF, since the small molecular weight urea reaches complete equilibrium across the capillary-alveolar membrane. The ratio between BALF-urea and serum urea allows the calculation of the dilution of the original volume of ELF by BALF saline. This ratio can be used to calculate the individual constituents of ELF. (Rennard *et al.* 1986).

Various soluble constituents in BALF, including cytokines and other mediators of inflammation, collagen metabolism markers, fibronectin, ACE, etc, have been measured for scientific purposes, although none of them have yet reached the status of a standard diagnostic method.

2.7. Collagens and procollagens in immunohistochemical lung specimens

2.7.1. Normal lung

Mature collagen I is located in alveolar septa, whereas collagen III is mainly occurs in capillary walls and aggregates of collagen in healthy normal lung. Collagen IV and V are located in alveolar and capillary basement membrane. (Madri & Furthmayr 1980). Collagen I has also been detected in pleural, peribronchial and perivascular connective tissue as well as in bronchial mucosa and subintima. Collagen III is expressed mainly in the latter two locations. (Bateman *et al.* 1981). Immunoreactivity of procollagen I carboxyterminal propeptide has not been detected in normal lung specimens (McDonald *et al.* 1986, Roman *et al.* 1995).

2.7.2. Sarcoidosis

Sarcoidosis granulomas have been shown to include interstitial collagens I and III. In the early stage of the disease, both collagens have been detected peripherally, whereas in mature and late stages they are also seen around and within granulomas (Peyrol *et al.* 1986, Roman *et al.* 1995). PICP immunoreactivity has been shown to be distributed within multinucleated giant cells, macrophages and surrounding fibroblasts (Roman *et al.* 1995). Granulomas from tuberculosis and sarcoidosis skin biopsy specimens have been found to contain PICP positive cells showing fibroblast morphology and being scattered throughout the granuloma core. Accentuated staining for the extracellular matrix has been seen both in granulomas and in the peri-granulomatous regions. (Marshall *et al.* 1996).

2.7.3. Fibrosing alveolitis

The mean percentage of type I collagen appears to be higher than that of type III collagen in autopsy samples from patients who died of fibrosing alveolitis as compared to open lung biopsies and controls (Kirk *et al.* 1984). In fibrotic lung, collagen I has been shown to be markedly increased throughout the thickened septa, whereas collagen III is absent from the septa, although detectable in vascular structures (Madri & Furthmayr 1980). Collagen I forms an irregular pattern in all zones of fibrosis, whereas collagen III is less intensively stained in some areas (Takiya *et al.* 1983). Bateman *et al.* (1983) has shown that collagen I predominates at all sites of fibrosis, and in areas where the fibre bundles are closely packed, collagen I stains are weaker and collagen III is not detectable. Some fibrosis patients show collagen III at subepithelial sites or in loosely arranged fibrils between densely packed collagen I fibres in alveolar walls. These collagen III-positive patients deteriorate in follow-up. (Bateman *et al.* 1983).

Collagen I mRNA has been detected in acute interstitial pneumonitis of Hamman-Rich type, bleomycin-induced fibrosis, bronchitis obliterans organising pneumonia and fibrosing alveolitis. Collagen I protein can only be detected in fibrosing alveolitis, whereas both collagen III and IV and their mRNA have been observed in all of the abovementioned disorders. (Specks *et al.* 1995).

In biopsies obtained from patients with active fibrosis, anti-PICP antibodies stained intensely both interstitial and alveolar fibroblasts, whereas patients with quiescent disease show no immunoreactivity (McDonald *et al.* 1986). Kuhn *et al.* (1989) demonstrated that only 13/22 patients with fibrosing alveolitis show collagen-synthesising fibroblasts with PICP positivity, and the extent of staining varied within 1-20%. Immunoreactive fibroblasts form subepithelial clusters (fibroblastic foci) near the air-tissue interface, and the cells closer to the air-tissue interface are larger and more intensively stained than the deeper cells. The cells comprising these clusters are spindle-shaped and usually arrayed parallel to one another. Active synthesis of type I procollagen in these foci has been shown by the myofibroblasts, which are α -smooth muscle actin-positive fibroblast-like cells with contractile filament-laden stroma. (Kuhn & McDonald 1991).

Procollagen I aminoterminal propeptide has been detected in myofibroblasts of fibroblast foci with relatively little mature collagen compared to other areas (Bensadoun *et al.* 1996).

2.8. Procollagen propeptides in bronchoalveolar lavage fluid and serum in diffuse parenchymal lung diseases

Several works have examined the role of procollagen III aminoterminal propeptide in bronchoalveolar lavage fluid and serum as a marker of inflammation, fibrosis or disease prognosis both in sarcoidosis and fibrosing alveolitis. However, the results of these studies are contradictory. Especially the information on the role of serum measurements and the role of BALF-PIIINP as a prognostic marker has been diverging. All but one of the previous studies on PIIINP in BALF have been performed with bovine antibodies, and there has been a variety of concentration methods from no concentration at all to 100 fold concentration before analysing PIIINP in BALF (Table 1). In alveolar lavage fluid, the concentrations of PIIINP have been low in healthy volunteers (Sutinen *et al.* 1995).

The information available on procollagen I carboxyterminal propeptide in BALF and serum is limited to two studies on sarcoidosis and cryptogenic fibrosing alveolitis (Bacchella *et al.* 1996, Tukiainen *et al.* 1994). Serum PICP has been investigated in one study with systemic sclerosis (Kikuchi *et al.* 1994).

Table 1. Comparison of methods for determining BALF-PIIINP in previous studies.

Source	Antibody	Disease	Concentration of the BALF	Adjusted to
Low <i>et al.</i> 1983	Bovine (Hoechst®)	Sarcoidosis Fibrosing alveolitis	50x Pressure filtration	Protein
Bjermer <i>et al.</i> 1986	Bovine (Hoechst®)	Sarcoidosis	Not concentrated	Albumin
Bjermer <i>et al.</i> 1987a	Bovine (Hoechst®)	Sarcoidosis	Not concentrated	Not adjusted
O'Connor <i>et al.</i> 1989	Not mentioned	Sarcoidosis	20x Ultrafiltration	Protein
Bjermer <i>et al.</i> 1991	Bovine (Hoechst®)	Sarcoidosis	Not concentrated	Not adjusted
Straub <i>et al.</i> 1995	Bovine (Behringwerke®)	Sarcoidosis	Not concentrated	Not adjusted
Milman <i>et al.</i> 1995	Human (Farnos- Diagnostica®)	Sarcoidosis	Not concentrated	Not adjusted
Schaberg <i>et al.</i> 1994	Bovine (Behringwerke®)	Sarcoidosis Fibrosing alveolitis Tuberculosis Bronchial carcinoma Lymphangitis carcinomatosa	50-60x Dialysing, Ultrafiltration	Not adjusted
Cantin <i>et al.</i> 1988	Bovine (Behringwerke®)	Fibrosing alveolitis	10x Ultrafiltration	Not adjusted
Bjermer <i>et al.</i> 1989	Bovine (Behringwerke®)	Fibrosing alveolitis	Not concentrated	Not adjusted
Low <i>et al.</i> 1992	Bovine (Behringwerke®)	Fibrosing alveolitis	10-100x Ultrafiltration	Not adjusted
Harrison <i>et al.</i> 1993	Bovine (Hoechst®)	Fibrosing alveolitis	Not concentrated	Not adjusted
Fujimoto <i>et al.</i> 1995	Bovine (Behringwerke®)	Fibrosing alveolitis Sarcoidosis Collagen vascular dis- order	50x Ultrafiltration	Not adjusted
Kuroki <i>et al.</i> 1995	Bovine (Behringwerke®)	Fibrosing alveolitis	Not concentrated	Not adjusted

2.8.1. Sarcoidosis

The serum levels of procollagen III aminoterminal propeptide have been found to be elevated in sarcoidosis patients compared to controls (Bacchella *et al.* 1996, Luisetti *et al.* 1990, Schoenfeld *et al.* 1996). The initial level of S-PIIINP has been shown to correlate with serum angiotensin-converting enzyme, but not with other disease activity parameters, such as ^{67}Ga uptake, BALF-lymphocyte percentage, vital capacity or diffusion capacity. The S-PIIINP level fails to characterise sarcoidosis patients with a fibrotic radiological pattern and is unable to predict poor prognosis. (Luisetti *et al.* 1990). In contrast, another study showed serum PIIINP to be higher in progressive than stable sarcoidosis. In this particular study steroid medication resulted in a significant decrease in S-PIIINP during five years' follow-up, and serial measurements of serum levels of PIIINP, but not S-ACE, correlated with the clinical course. S-PIIINP and S-ACE did not correlate with each other in this study. (Pohl *et al.* 1992). S-PIIINP has been found to have a negative correlation with vital capacity and total lung capacity and a positive correlation with S-ACE (Schoenfeld *et al.* 1996). On the other hand, no difference has been detected between the levels of PIIINP in the sera of sarcoidosis patients and controls, nor any correlation with disease activity (Poole *et al.* 1989, Milman *et al.* 1995).

Low and colleagues (1983) published the first study concerning procollagen III aminoterminal propeptide in bronchoalveolar lavage fluid. They found clear differences in the levels of BALF-PIIINP between controls and sarcoidosis patients, whereas no corresponding differences were seen in the serum levels. The highest BALF levels were found in fibrosing alveolitis. They detected a poor correlation between BALF-PIIINP and the clinical severity of the disease. (Low *et al.* 1983). PIIINP in BALF has been shown to be more markedly elevated in patients with sarcoidosis, fibrosing alveolitis and lymphangitis carcinomatosa than in those with bronchial cancer, tuberculosis and pneumonia (Schaberg *et al.* 1994). The level of PIIINP in BALF has shown significant inverse correlations with vital capacity, forced expiratory volume and diffusion capacity, appearing to be related to radiological pulmonary findings, and with S-ACE and to correlate poorly with lavage cell profiles, except for mast cells (Bjermer *et al.* 1986, Bjermer *et al.* 1987b). On the contrary, BALF-PIIINP has been suggested to be a marker of active sarcoidosis comparable to the cell differentiation count and the CD4/CD8 T-lymphocyte ratio in BALF (Straub *et al.* 1995). Elevated BALF-PIIINP has also been detected in sarcoidosis patients whose endobronchial biopsies show sarcoidosis changes and whose clinical course is progressive (Bjermer *et al.* 1991). In a follow-up study of 12 months, the initial level of PIIINP in BALF did not correlate with the severity of the disease as assessed by lung function testing, and it was therefore concluded that PIIINP synthesis is rather associated with the inflammation than an early sign of the development of a chronic disease (O'Connor *et al.* 1989).

On the other hand, Milman *et al.* (1995) showed no statistically significant differences between sarcoidosis and controls in the levels of S- or BALF-PIIINP. Cantin *et al.* (1988) were unable to detect significant differences in the PIIINP levels in BALF between sarcoidosis and controls. Furthermore, no difference has been shown between clinically active and inactive disease in newly diagnosed sarcoidosis. However, patients who deteriorated during a follow-up of 3 years had initially higher levels of BALF-PIIINP than patients showing spontaneous remission. (Selroos *et al.* 1994).

In a preliminary study, Tukiainen and co-workers reported that patients who did not recover completely had similar levels of PIIINP but elevated PICP in BALF compared with those who recovered during three-year follow-up. Patients treated due to advanced parenchymal changes had higher levels of PIIINP and PICP in BALF than those without treatment. BALF-lymphocytes had a positive correlation with both procollagen peptides. (Tukiainen *et al.* 1994). The levels of PICP and PIIINP in the serum of newly diagnosed sarcoidosis patients have been shown to be elevated compared with controls, although they do not correlate with each other. PICP does not identify patients with parenchymal fibrotic changes in chest radiographs. (Bacchella *et al.* 1996).

2.8.2. Fibrosing alveolitis

Procollagen III aminoterminal propeptide in bronchoalveolar lavage fluid and serum has been shown to be elevated in fibrosing alveolitis (Harrison *et al.* 1993). On the other hand, serum levels of PIIINP were found to increase in a small series of primary lung cancer, pulmonary tuberculosis, and chronic bronchitis patients, whereas bronchial asthma and fibrosing alveolitis patients did not differ significantly from healthy controls (Watanabe *et al.* 1985). PIIINP in BALF but not in serum has been observed to be elevated in fibrosing alveolitis compared with sarcoidosis and controls, but to lack correlation with disease activity (Low *et al.* 1983). However, the same group of investigators later concluded that clinical, radiological, and physiologic scoring of disease severity may correlate with serum PIIINP. BALF-PIIINP, when normalised to albumin, is also higher than S-PIIINP in fibrosing alveolitis patients and the healthy volunteers consistently with local pulmonary production. BALF-PIIINP was elevated in fibrosing alveolitis compared to controls, whether expressed as a concentration or normalised to albumin. (Low *et al.* 1992).

PIIINP in BALF has been shown to correlate with an increased ability of BALF to stimulate fibroblast proliferation in fibrosing alveolitis, but not in sarcoidosis or controls (Cantin *et al.* 1988). It appears that patients with deteriorating chest radiographs and lung function have higher PIIINP in BALF than those with a stable course (Bjermer *et al.* 1989). Eosinophilic cationic protein (ECP) and PIIINP in BALF may help to discriminate acute and rapidly progressive disease from stable or less active disease (Fujimoto *et al.* 1995). Elevated levels of PIIINP in BALF have correlated with the levels of interferon- γ in BALF (Kuroki *et al.* 1995).

Both BALF-PIIINP and -PICP were elevated compared with controls, and PICP in BALF was also elevated compared with sarcoidosis in fibrosing alveolitis (Tukiainen *et al.* 1994). No other studies on PICP in BALF or serum have been published.

Various collagen diseases have multiple pulmonary manifestations, one of them being fibrosing alveolitis. According to one study, serum PICP is elevated in diffuse scleroderma compared with localised disease. Patients with elevated serum PICP had significantly more joint and pulmonary changes compared to those with normal PICP. (Kikuchi *et al.* 1994). Both elevated serum (Diot *et al.* 1995) and BALF (Harrison *et al.* 1990) levels of PIIINP have correlated with computed tomography findings of the lungs. In addition, during cyclophosphamide medication for systemic sclerosis the serum PIIINP

level has been shown to decrease (Åkesson *et al.* 1993). In established rheumatoid lung disease, the BALF-PIIINP level is elevated with mild pulmonary involvement or controls (Gilligan *et al.* 1990).

2.8.3. Asbestos-related pulmonary disorders and other pneumoconiosis

The role of serum PIIINP as a biomarker of any pneumoconiosis is unclear (Borm 1994). The information on the concentration of procollagen I carboxyterminal propeptide in BALF, epithelial lining fluid or serum in asbestos-exposed individuals is very limited. There is one abstract that shows an elevated level of PICP in BALF in patients with asbestos-induced pleural and/or mild parenchymal fibrosis. The same study shows no statistical difference in procollagen III aminoterminal propeptide in BALF between individuals exposed to asbestos and controls. (Tukiainen *et al.* 1994).

Very few studies have been conducted on PIIINP following work-related asbestos exposure. Most of the studies are experimental works on asbestos-exposed sheep. An increase of type III collagen in histological investigation of lung tissue and an increase of PIIINP in BALF after two months of chrysotile exposure have been shown in sheep (Begin *et al.* 1987b).

The sheep with abnormal chest radiography findings after 18 months' exposure to chrysotile had significantly elevated levels of PIIINP in BALF (Begin *et al.* 1990). A study on crocidolite-exposed rats showed that type III collagen is increased first, which increase is later followed by an increase of type I collagen (Arden & Adamson 1992). These experimental exposures have, however, used high and toxic concentrations of asbestos fibres. In humans, the aminoterminal propeptide of type III procollagen was significantly elevated in BALF in subjects with asbestosis and asbestos-associated alveolitis compared with asbestos-exposed workers without the disease (Begin *et al.* 1986). Another study on serum PIIINP in workers exposed to asbestos fibres suggests that serum PIIINP might be a useful index for the early diagnosis of asbestos-induced pulmonary fibrosis (Cavalleri *et al.* 1988).

Silica-exposed workers had elevated PIIINP in BALF. This change was only seen in progressive disease. (Begin *et al.* 1987a). In coal workers' pneumoconiosis, serum PIIINP was not a marker of interstitial or respiratory effects of coal dust (Schins *et al.* 1995). Furthermore, serum PIIINP did not predict the development of pneumoconiosis in coal workers during five years of follow-up (Schins & Borm 1994).

2.8.4. Other parenchymal disorders

Farmer's lung and other forms of hypersensitivity pneumonitis may lead to interstitial fibrosis. The susceptibility of an individual to develop lung fibrosis is variable. The level of procollagen III aminoterminal propeptide in BALF rises in an acute attack of hypersensitivity pneumonia and decreases during the follow-up to a slightly elevated or normal level (Bjermer *et al.* 1987a, Larsson *et al.* 1992, Cormier *et al.* 1993, Teschler *et al.* 1993). Asymptomatic farmers have normal levels of BALF-PIIINP (Larsson *et al.* 1988,

Larsson *et al.* 1992). Serum PIIINP has been shown to remain at the control level in most patients at the acute stage of the disease followed by a small but significant increase after six months (Anttinen *et al.* 1986).

After radiation therapy of the lungs, serum PIIINP does not show consistent changes or correlate with radiation fibrosis as assessed by computed tomography scans of the lungs (Maasilta *et al.* 1991). Combination chemotherapy including bleomycin leads to a decline in lung function and causes an increase of PIIINP in serum within two months (Villani *et al.* 1992).

The adult respiratory distress syndrome (ARDS) is a severe life-threatening organ failure, which is characterised by tachypnoea, hypoxemia, diffuse interstitial infiltrates, alveolar oedema, and loss of lung compliance (Ashbaugh *et al.* 1967). It is a stereotypical response of the lungs to a variety of insults, such as pneumonia, gastric contents aspiration, consequences of systemic disorders, e.g. septic shock and multiorgan trauma, and inhalation of toxins, that affects previously healthy individuals and portends a poor outcome. Pulmonary fibrosis and sepsis are the major causes of death in patients with advanced ARDS. The results concerning BALF- and serum PIIINP in ARDS are divergent. The level of PIIINP in serum and BALF (Farjanel *et al.* 1993), or in serum but not in BALF (Hällgren *et al.* 1989), has been shown to be elevated in patients with ARDS. Also, the level of PIIINP in BALF is elevated in patients who develop intra-alveolar fibrosis (Farjanel *et al.* 1993). In addition, recent studies have shown that high concentrations of PIIINP in BALF (Clark *et al.* 1995) or in oedema fluid related to ARDS (Chesnut *et al.* 1997) predict a poor prognosis. Plasma procollagen I aminoterminal propeptide and PIIINP levels are elevated on the first day of ARDS and, if they still remain elevated one week after the onset of the disease, they predict a poor outcome. This same study indicated that treatment with cortisone causes a rapid and sustained reduction of BALF and plasma PINP and PIIINP as well as other disease activity markers in ARDS. (Meduri *et al.* 1998).

3. Purpose of the present study

The activity of interstitial lung diseases and the prediction of disease progression is difficult to assess and poorly understood. The prognosis of sarcoidosis is good in most cases, and only a small number of sarcoidosis patients develop progressive pulmonary fibrosis. However, no accurate markers for the assessment of the activity of sarcoidosis are available. In contrast, the prognosis of fibrosing alveolitis is poor in most cases, leading to severe impairment of respiratory function and finally to death. Apart from open or thorascopic lung biopsies with typical histopathology, assessment of the disease activity and prognosis is difficult. Exposure to asbestos may lead to progressive fibrosis of the lungs, asbestosis, after a latency of 15-20 years with a poor prognosis and no effective cure.

The present study was undertaken to characterise the role of procollagen I and III propeptides in various interstitial lung diseases to examine their role as diagnostic and prognostic tools in these diseases and as markers of fibrogenesis.

The specific aims of the study were as follows:

1. to apply new tools to investigating collagen synthesis in interstitial lung diseases for purposes of clinical practice.
2. to evaluate the role of type I and III procollagen markers in serum and BALF in disease activity and the prognosis of sarcoidosis, fibrosing alveolitis and asbestos-related diseases.
3. to compare procollagen propeptide levels in BALF, serum and in epithelial lining fluid (ELF) to estimate the level of active inflammation.
4. to assess the accumulation and distribution of type I and III procollagens and the corresponding pN-collagens in histological specimens in fibrosing alveolitis and sarcoidosis.

4. Materials and methods

4.1. Study population

The study included 160 patients investigated because of suspected interstitial lung disease or asbestos exposure. Altogether 137 consecutive patients were admitted to the pulmonary departments of Päivärinne Hospital (Muhos, Finland) between February 1990 and August 1992 (I-III). The patients came from the province of Oulu in Northern Finland and had been referred to investigations mostly by general practitioners. These 137 patients underwent bronchofiberscopy and bronchoalveolar lavage due to their symptoms and findings. The study was performed as part of the normal diagnostic procedures and the serum and BALF samples were split off from routine specimens. The clinical data, including the laboratory results and pulmonary function tests, were obtained from the case histories. The remaining 23 patients had undergone open lung biopsies between 1981 and 1998 (IV). Pulmonary specialists from the pulmonary clinics of Northern Finland had referred these patients to open lung biopsies. Their paraffin blocks of biopsy specimens were obtained from the pathology files at the Department of Pathology, University of Oulu, Finland, and the clinical data were obtained from the case histories.

Sixty patients out of the 137 were diagnosed as having sarcoidosis (I). Forty of them had non-parenchymal sarcoidosis (Stage 0-I in chest radiography), and 20 were considered to have parenchymal involvement (Stage II-III in chest radiography). No visible changes in radiography were considered stage 0. Nodular hilar enlargement was the criterion for hilar adenopathy (Stage I). Reticular, nodular and/or linear patterns were criteria for parenchymal involvement (Fraser *et al.* 1994). All the patients in the former group were newly diagnosed, whereas five patients in the latter group had been formerly diagnosed as having sarcoidosis and two of them were on oral corticosteroid medication at the time of diagnosis. Histological confirmation of diagnosis was available for 35 patients with biopsies taken either transbronchially, via mediastinoscopy, from superficial lymph nodes or from the skin. Typical sarcoidosis changes were seen in 16 of the parenchymal and 19 of the non-parenchymal patients. In the remaining 25 cases the diagnosis was based on a typical clinical picture, marked lymphocytosis in BALF and typical findings in chest radiography as well as exclusion of other diseases. The clinical characteristics of the sarcoidosis patients are presented in table 2.

Fibrosing alveolitis was diagnosed in 18 of the 137 patients (II). Biopsies in 9 patients and autopsy in one patient confirmed the diagnosis. The diagnosis of the remaining 8 patients based on typical findings in chest radiography, BALF, spirometry and diffusion capacity, and the exclusion of other pulmonary diseases. Sixteen of these 18 patients were considered to have idiopathic fibrosing alveolitis, one patient had rheumatoid arthritis and one had scleroderma with pulmonary involvement. The mean follow-up time of these patients was 6 years and 2 months (range between 5 and 7 years). The clinical characteristics of the fibrosis patients are shown in table 2.

Table 2. Clinical characteristics of the patients with sarcoidosis and fibrosing alveolitis and the controls (I-II).

		Sarcoidosis	Fibrosing alveolitis	Controls
Subjects	n	60	18	17
Age	yrs	39 ± 9.5	61 ± 7.7	47 ± 9.1
Sex	M / F	39 / 29	5 / 13	9 / 8
Height	cm	172 ± 7.8	159 ± 7.0	165 ± 7.9
Weight	kg	77.3 ± 13.7	72 ± 15.9	73 ± 15.3
FEV1	l	3.8 ± 1.0	2.21 ± 0.5	3.17 ± 0.43
	%pred	95 ± 16.2	83 ± 9.4	93 ± 11
FVC	l	4.7 ± 1.2	2.68 ± 0.7	4.02 ± 0.66
	%pred	94 ± 14.4	81 ± 10.4	97 ± 10.1
DLCO	mmol/min/kPa	9.9 ± 2.3, n=57	4.89 ± 1.7	9.04 ± 2.0 (n=16)
	%pred	102 ± 16.6	71 ± 21.8	106 ± 19.6
DLCO/VA	mmol/min/kPa/l	1.67 ± 0.3, n=57	1.30 ± 0.33	1.68 ± 0.3 (n=16)
	%pred	106 ± 12.4	88 ± 20.7	108 ± 17.8
Recovery of BALF	ml	136 ± 12.0	131 ± 11.3	128 ± 14
BALF-albumin	mg/ml	113 ± 111	63.9 ± 31.3	42.2 ± 17.4
BALF-neutrophils	%	2.3 ± 3.3	10.5 ± 8.8	3.2 ± 6.0
BALF--lymphocytes	%	37.9 ± 18.0	16.6 ± 14.1	18.1 ± 13.1
BALF--eosinophils	%	1.4 ± 1.5	1.1 ± 2.4	0.1 ± 0.5
Smokers	n%	14 (23)	7 (39)	5 (29)
Ex-smokers	n%	18 (30)	3 (17)	4 (24)
Nonsmokers	n%	28 (47)	8 (44)	8 (47)

Forty-two patients were referred to investigations due to a history of occupational asbestos exposure and changes in chest radiography (III). The radiological changes included either pleural plaques or an interstitial basal reticular-linear pattern suggestive of fibrosis and asbestosis. Five patients were diagnosed as having asbestosis, 25 had pleural plaques, and the chest radiographs of 12 patients were considered normal. In one patient the asbestosis diagnosis was based on an open lung biopsy. The remaining 4 patients with asbestosis had typical findings in chest radiography, spirometry and diffusion capacity and a significant but variable amount of asbestos bodies in BALF. Other pulmonary diseases were excluded. The mean follow-up time was 7 years (from 5 years 7 months to 7 years 10 months). The characteristics of the asbestos-exposed individuals are presented in table 3.

Table 3. Clinical characteristics of the patients exposed to asbestos fibres (III).

		Asbestosis	No parenchymal involvement
Subjects	n	5	37
Age	yrs	55 ± 9.6	54 ± 8.0
Sex	M / F	5/0	37/0
Height	cm	171 ± 8.0	171 ± 7.3
Weight	kg	76 ± 7.4	78 ± 13.2
FEV1	l	2.76 ± 0.7	3.52 ± 0.7
	%pred	74 ± 23	95 ± 22
FVC	l	3.59 ± 0.9	4.44 ± 0.75
	%pred	78 ± 23	95 ± 10
DLCO	mmol/min/kPa	5.48 ± 1.2	9.53 ± 1.61 (n=33)
	%pred	60 ± 10	104 ± 15
DLCO/VA	mmol/min/kPa/l	1.26 ± 0.5	1.59 ± 0.27 (n=31)
	%pred	84 ± 28	107 ± 17
Recovery of BALF ml		119 ± 8.9	125 ± 17.9
BA mg/ml	mg/ml	39.2 ± 28.7	54.9 ± 21.5
BALF-neutrophils	%	2.0 ± 2.2	3.2 ± 4.0
BALF--lymphocytes	%	15.0 ± 14.6	15.0 ± 12.3
BALF--eosinophils	%	2.2 ± 2.7	1.5 ± 1.7
Smokers	n%	4 (75)	9 (24)
Ex-smokers	n%	0	13 (35)
Nonsmokers	n%	1 (25)	15 (41)

The control group (I-II) consisted of 17 subjects who underwent fiberoptic bronchoscopy because of prolonged cough, haemoptysis or other minor respiratory symptoms. Four of the patients were later considered asthmatic. There were no changes in the chest radiographs of this group. The characteristics of the control group are shown in table 2.

The Ethics Committee of Päivärinne Hospital approved the research protocol. Informed consent was obtained from each subject beforehand.

Twenty-three patients who had undergone open or thoracoscopic lung biopsy (IV) were drawn from the files of the Department of Pathology, University of Oulu. Histopathologically typical 10 cases with fibrosing alveolitis and 13 cases with sarcoidosis were re-evaluated. The diagnoses of the patients were based on light-microscopic evaluations using the previously described histological criteria (Dail & Hammer 1994, Thurlbeck & Churg 1995, Katzenstein 1997). The patients' histological biopsies were investigated immunohistochemically. The clinical characteristics of the sarcoidosis patients are presented in table 4 and those of the fibrosing alveolitis patients in table 5.

Table 4. Characteristics of the patients with sarcoidosis in the immunohistochemical study (IV).

N:o	Age	Sex	Weight kg	Height cm	Smoking	Post biopsy treatment	ACE U/l	FEV	FVC	DLCO	DLCO/VA %pred
1	26	F	40	144	Non	None	29*	73	81	74	91
2	34	M	70	183	Ex	Cortisone	120	99	91	100	120
3	37	M	70	179	Non	None	182	94	96	112	120
4	27	M	57	180	Current	Cortisone	112	98	104	71	77
5	55	F	65	162	Non	Cortisone	95	77	78	70	98
6	44	F	62	167	Non	Cortisone	192	95	103	98	100
7	69	F	61	153	Non	Cortisone	118	95	105	76	74
8	55	F	73	166	Non	Cortisone	113	118	124	97	87
9	57	F	67	160	Non	None	63	84	80	96	117
10	35	F	59	162	Non	Cortisone	122	64	78	64	91
11	58	M	69	171	Non	None	95	41	53	87	113
12	52	F	57	162	Non	Cortisone	**	77	84	NA	NA
13	45	M	77	185	Current	None	197	99	103	70	72

ACE: Angiotensin-converting enzyme, reference level 34-160 U/l ; NA: data not available; Non: Nonsmoker; Ex: Ex-smoker; Current: Current smoker. *: Reference level 14-46 U/l; **: ACE-inhibiting medication.

Table 5. Characteristics of the patients with fibrosing alveolitis in the immunohistochemical study (IV).

N:o	Age	Sex	Weight kg	Height cm	Smoking	Follow-up months	Post-biopsy treatment	Status at the end of follow-up	FEV1 %pred	FVC %pred	DLCO %pred	DLCO/ VA %pred
1	59	M	84	167	Current	36	None	NA	92	100	36	42
2	57	F	82	165	Non	115	Cort	FA	85	76	64	79
3	66	F	90	164	Non	16	Cort, Aza	FA	44	43	38	70
4	43	F	77	165	Non	176	Cort, Cyclo	Alive	83	85	36	50
5	58	F	74	166	Non	45	Cort	Alive	65	66	83	102
6	48	F	74	164	Non	26	Cort, Cyclo	FA	59	52	87	135
7	44	M	89	185	Current	7	Cort	FA	80	71	57	73
8	66	F	85	163	Non	40	None	Alive	88	85	77	80
9	63	F	69	157	Ex	43	None	Alive	81	81	67	75
10	73	M	90	172	Non	35	Cort	Alive	63	60	NA	NA

NA: data not available; FA: died of fibrosing alveolitis; Non: Nonsmoker; Ex: Ex-smoker; Current: Current smoker; Cort: Cortisone; Aza: Azathioprine; Cyclo: Cyclophosphamide.

4.2. Methods

The chest radiography included posteroanterior and lateral views. A physician specialized in respiratory medicine interpreted them first. The radiographs were then interpreted retrospectively and blindly by an experienced thoracic radiologist.

The pulmonary function tests included forced expiratory volume in one second (FEV1) and forced vital capacity (FVC), which were measured with a flow-volume spirometer. Diffusion capacity (DLCO) and the specific diffusion coefficient (DLCO/VA) were analysed by the single-breath technique.

Fiberoptic bronchoscopy for sampling BALF was performed under local anaesthesia with lignocain. The patients were premedicated with diazepam and atropine. A broncho-fibroscope was wedged into the right middle lobe or the left lingula. Saline solution was then installed in 10 aliquots of 20 ml. After gentle aspiration, the recovered fluid was collected in a plastic bottle and kept on ice. After centrifugation (400xg for 15 min), 30 ml of the cell-free supernatant was stored for later assays.

4.2.1. Bronchoalveolar lavage and serum (I-III)

The serum angiotensin-converting enzyme was analysed with a method modified for the Kone Specific analyser (Kone Oy, Espoo, Finland) (Harjanne 1984). Interleukin-2-receptor was analysed with enzyme-linked immunosorbent assay (ELISA) (DAKO A/S, DK 2600 Glostrup, Denmark). Serum and BALF-urea and albumin and serum alanine aminotransferase (ALAT) were assayed with routine laboratory methods.

The total number of cells in BALF was counted in a Bürger haemocytometer, and their viability was assessed by trypan blue exclusion. 1 ml of lavage fluid was fixed in an equal volume of 95% ethanol, and Millipore filter preparations were made with Papanicolaou staining. Cytocentrifuge preparations (700 rpm, 5 min; Cytospin 2, Shandon Instruments, Astmoor, UK) of unfixed cells (roughly 100.000 cells/slide) were air-dried overnight at room temperature and stained with May-Grünwald-Giemsa. For differential counts, 200 cells were counted on both filter and cytocentrifuge preparations.

The amount of epithelial lining fluid (ELF) was calculated with the urea method (Renard *et al.* 1986).

The number of asbestos bodies in BALF was determined from Millipore filter and cytocentrifuge (Cytospin 2, Shandon Instruments, Astmoor, UK) preparations. Two or three filter preparations containing 5 ml of lavage fluid each and four or five cytocentrifuge preparations containing 0.4 ml of lavage fluid each were prepared as described above. Both the Millipore filter and the cytocentrifuge preparations were stained with Pearl's iron stain. The filter preparations were mounted on glass slides, and both the filter and the cytocentrifuge preparations were covered with coverslips. Asbestos bodies were identified on the basis of the previously described criteria (Churg & Warnock 1981) using a light microscope with a 20x objective. The number of asbestos bodies was expressed as per ml of BALF.

4.2.1.1. Radioimmunoassays for procollagen I carboxyterminal and procollagen III aminoterminal propeptides in bronchoalveolar lavage and serum (I-III)

Serum procollagen I carboxyterminal propeptide (Melkko *et al.* 1990) and procollagen III aminoterminal propeptide (Risteli *et al.* 1988) were assayed by commercial radioimmunoassay kits (Orion Diagnostica Oy, Oulunsalo, Finland) employing human antigen and specific polyclonal antibodies.

For assaying PIIINP and PICP in bronchoalveolar lavage fluid by radioimmunoassay, the original methods for serum were modified in such a way that no concentration or further processing of the sample was necessary. The BALF samples used were bigger (1 ml) than the samples used in the serum methods (200 μ l with PIIINP and 100 μ l with PICP), and the tracer and antiserum amounts were, respectively, smaller but more concentrated compared to the original method. Human antigens were employed to induce polyclonal anti-PIIINP and anti-PICP antibodies. In the BALF-PIIINP assay, 1 ml of BALF or appropriate standard, adjusted to 0.1M phosphate buffer, was incubated with 200 μ l of 125 I-PIIINP tracer solution diluted 1:1000 with phosphate-buffered saline containing 0.04 % Tween -20 (PBS/Tween) and 100 μ l of antiserum (diluted 1:1000 with PBS/Tween) at 37 °C. After 2 h incubation, 1.5 ml of secondary antibody (the original solid phase reagent concentrated x2) was added, and the tubes were further incubated at room temperature for 30 min and centrifuged at 3400 x g at 4 °C for 15 min, after which the radioactivity of the precipitates was counted. The standard curves were calculated using an automatic gamma counter (Clinigamma 1272, Wallac, Turku, Finland). The BALF-PICP assay was performed similarly: 200 μ l of the 125 I-PICP tracer solution (dilution 1:2500) and 150 μ l of the antiserum (dilution 1:1000) were incubated with the samples, after which the immunocomplex was precipitated with 1 ml of secondary antibody (the original solid concentrated x2). The results are expressed either as μ g of propeptide per liter of recovered BALF or as μ g of propeptide per liter of ELF. The sensitivities of the original methods are 0.2 μ g/l with 200 μ l serum sample for PIIINP and 1.2 μ g/l with 100 μ l serum sample for PICP. The intra- and interassay variations of the original methods for PIIINP are 4.3% and 5.3% and those for PICP 3.2% and 6.6 %, respectively.

4.2.2. Immunohistochemical methods (IV)

Open lung biopsies were taken from different parts of the left or right lung. The biopsy material was fixed in 10% formalin under vacuum in order to expand the tissue and to remove air bubbles (Wagenvoort 1980) or perfused by injecting fixative into bronchioles, using a small syringe (Dail & Hammar 1994). The specimens were then dehydrated and embedded in paraffin. 4 μ m sections were stained with hematoxylin-eosin, Giemsa, Verhoeff, van Gieson, Pearl's iron, periodic acid-Schiff alcian blue and periodic acid-Schiff stains. Uninvolved peripheral lung tissue was obtained from five patients operated on for pulmonary malignancy and used as controls.

Primary human polyclonal antibodies for procollagen I aminoterminal propeptide (Melkko *et al.* 1996) and procollagen III aminoterminal propeptide (Risteli *et al.* 1988) were used. For immunohistochemistry, 4 μm serial sections were deparaffinized and pre-treated with 0.4% pepsin for 30 minutes at 37°C. Endogenous peroxidase activity was blocked by incubating the slides in 0.3% H_2O_2 in absolute methanol for 30 minutes. The tissue sections were incubated with 2% goat serum for 10 minutes. Primary antibodies for PINP and PIIINP were used in concentrations of 1:100 and 1:50, respectively. The sections were incubated with primary antibody at 4 °C overnight, followed by a biotinylated goat secondary antibody (at dilution of 1:300 for 10 minutes) and the avidin-biotin-peroxidase complex (both from Dakopatts, Glostrup, Denmark). The colour was developed with diaminobenzidine. The sections were counterstained with a light hematoxylin stain and mounted with Eukitt (Kindler, Frieburg, Germany). The negative control stain consisted of substituted phosphate-buffered saline (PBS) at pH 7.2.

4.3. Statistical analysis

Non-parametric tests were used in the statistical evaluation (I-III). The correlations were assessed with the Spearman correlation coefficient test (I-III). The differences between the groups were analysed with the Mann-Whitney U-test (I-III) or Fisher's exact probability test (II). P-values of 0.05 or less were considered significant. The statistical procedures were carried out using the SPSS for Windows® (SPSS Inc, Chicago, IL, USA).

5. Results

5.1. Procollagen I carboxyterminal propeptide and procollagen III aminoterminal propeptide in bronchoalveolar lavage fluid and serum of sarcoidosis and fibrosing alveolitis patients (I,II)

The levels of serum procollagen I carboxyterminal propeptide were similar in sarcoidosis, fibrosing alveolitis and the controls. Three sarcoidosis patients, one out of forty in the non-parenchymal and two out of twenty in the parenchymal sarcoidosis group, and none with fibrosing alveolitis had S-PICP over the upper normal limit of 200 µg/l. The levels of serum-PIIINP in the sarcoidosis and fibrosing alveolitis patients were similar and significantly higher than in the controls (Table 6). Eight sarcoidosis patients, five (12.5%) without parenchymal involvement and three (15%) with parenchymal changes, had elevated S-PIIINP compared to the normal upper limit of 4.5 µg/l. Four (22%) of the 18 fibrosing alveolitis patients had S-PIIINP above the normal upper limit. Two patients with fibrosing alveolitis had elevated S-ALAT values, suggesting the possibility of a hepatic lesion. These patients' values were excluded, since hepatic disease might elevate S-PIIINP. None of the controls had S-PIIINP or S-PICP over the reference values.

The levels of BALF-PIIINP were significantly elevated in sarcoidosis and fibrosing alveolitis compared to the controls, and BALF-PIIINP was significantly higher in sarcoidosis than in fibrosing alveolitis ($p < 0.05$, not shown). BALF-PIIINP was detectable in two (12%) of the 17 control subjects, in 22 (37%) of the 60 sarcoidosis patients, and in 9 (50%) of the 18 patients with fibrosing alveolitis. The concentration of PIIINP in ELF was higher in sarcoidosis than in fibrosing alveolitis ($p < 0.05$) and higher in the latter group than in the controls (Table 6). In the sarcoidosis patients the average concentration of PIIINP in ELF was 121 times and in the fibrosing alveolitis patients 13 times higher than the corresponding serum level.

The level of procollagen I carboxyterminal propeptide in BALF was elevated in fibrosing alveolitis and sarcoidosis compared to the controls. It was highest in fibrosing alveolitis, although the difference compared to sarcoidosis was not statistically significant. BALF-PICP was detectable in 22 (39%) out of 57 sarcoidosis and 7 (39%) out of 18 fibrosing alveolitis patients, while it could not be detected in the controls. The concentra-

tion of PICP in ELF was highest in fibrosing alveolitis, followed by the levels recorded in sarcoidosis, whereas no PICP in ELF was detectable in the controls (Table 6). The average concentration of PICP was twofold in ELF compared to the serum concentration in sarcoidosis and fourfold in ELF compared to serum concentration in fibrosing alveolitis.

Table 6. The concentrations of PIIINP and PICP in serum, BALF and ELF in the patients with sarcoidosis and fibrosing alveolitis and the controls (I-II).

		Sarcoidosis			Fibrosing alveolitis			Controls	
		Mean \pm SD	Median		Mean \pm SD	Median		Mean \pm SD	Median
S-PIIINP	$\mu\text{g/l}$	3.6 \pm 1.0	3.46	*	3.7 ⁺ \pm 1.1	3.6	*	2.7 \pm 0.9	2.5
S-PICP	$\mu\text{g/l}$	119 \pm 42.3	118.3		111.3 \pm 29.0	100.0		107.0 \pm 38.8	99.6
BALF-PIIINP	$\mu\text{g/l}$	5.8 \pm 9.9	1.17	***	1.9 \pm 6.1	0.1	*	0.1 \pm 0.5	0
BALF-PICP	$\mu\text{g/l}$	4.8 \pm 9.1#	0	**	13.6 \pm 25.9	5.4	**	0 \pm 0	0
ELF-PIIINP	$\mu\text{g/l}$	429 \pm 107.3	84.2	***	48.5 \pm 112.1	4.6	*	9.7 \pm 33.8	0
ELF-PICP	$\mu\text{g/l}$	246 \pm 447#	0	**	476.5 \pm 613.3	273.9	**	0 \pm 0	0

The significance of the difference from the controls was tested with the Mann-Whitney U-test. *: $p < 0,05$, **: $p < 0,01$, ***: $p < 0,001$. +Two patients' S-PIIINP values were excluded because of elevated S-ALAT values.

n=57.

5.2. Procollagen propeptide markers and other disease activity markers in sarcoidosis and fibrosing alveolitis (I, II)

In the sarcoidosis group, there was no correlation between S-PIIINP and BALF-PIIINP or S-PICP and BALF-PICP, while BALF-PICP and BALF-PIIINP correlated significantly (see Fig. 1 in paper I). Neither BALF-PIIINP nor BALF-PICP correlated with FVC or the specific diffusion coefficient. BALF-PIIINP correlated well with S-ACE, S-IL-2R, BALF-albumin and BALF-lymphocytes (see Fig. 1 in paper I), while BALF-PICP correlated only with BALF-lymphocytes and BALF-albumin and not with S-ACE or S-IL-2R. In the control group, the serum and BALF values of the procollagen markers did not correlate with each other, nor was there any correlation between these markers and BALF-albumin or lymphocytes.

Twenty of the 60 sarcoidosis patients with parenchymal sarcoidosis had higher S-ACE and BALF-PIIINP than the remaining 40 patients with non-parenchymal disease. There was no significant difference in S-IL-2R, S-PIIINP, S- and BALF-PICP, BALF-albumin or BALF-lymphocytes between these groups (Table 7).

Table 7. Levels of S-IL-2R, S-ACE, S-PIIINP, S-PICP, BALF-albumin, BALF-lymphocytes, BALF-PIIINP and BALF-PICP in non-parenchymal and parenchymal sarcoidosis (I).

Subjects	n	Non-parenchymal sarcoidosis		Parenchymal sarcoidosis	
		40		20	
S-ACE	U/l	139 ±59.1	(n=38)	190 ±91.6 *	(n=18)
S-IL2R	U/l	948 ±613	(n=34)	1537 ±1930	(n=16)
S-PIIINP	µg/l	3.5 ±1.0		3.6 ±1.1	
S-PICP	µg/l	116 ±37.3		127 ±51.1	
BALF-albumin	mg/l	111 ±124.3		120 ±81.3	
BALF-lymphocytes	%	35.8 ±18.5		42.1 ±16.7	
BALF-PIIINP µg/l	µg/l	4.4 ±9.3		8.7 ±10 **	
ELF-PIIINP	µg/l	251 ±533		785 ±1671 **	
BALF-PICP µg/l	µg/l	3.7 ±8.2	(n=37)	7.0 ±10.5	
ELF-PICP	µg/l	220 ±433		290 ±482	

The significance of the difference was tested with the Mann-Whitney U-test; * $p < 0.05$, ** $p < 0.01$

Forty-five of the 60 patients had prominent symptoms typical of sarcoidosis. BALF-PIIINP, BALF- PICP (see Table 4 in paper I), S-PIIINP and S-IL-2R ($p=0.001$, not shown) showed a tendency to increase in symptomatic disease. When the symptomatic patients were subdivided into ones with parenchymal and non-parenchymal disease, a tendency for the highest BALF-PIIINP and PICP values was observed in the groups of symptomatic patients with parenchymal disease (see Table 4 in paper I). Furthermore, S-PIIINP was significantly higher in symptomatic than non-symptomatic non-parenchymal sarcoidosis (symptomatic $3.77 \pm 0.96 \mu\text{gL}^{-1}$, non-symptomatic $2.89 \pm 0.77 \mu\text{gL}^{-1}$, $p < 0.01$, not shown). Lung function data did not differ significantly between non-parenchymal and parenchymal disease, nor between symptomatic and non-symptomatic patients with and without parenchymal changes (see Table 5 in paper I). Significantly higher levels of PIIINP were seen in the BALF samples which contained the highest percentage of mast cells (see Table 6 in paper I).

In fibrosing alveolitis, BALF-PICP ($r = -0.65$, $p < 0.01$) and ELF-PICP ($r = -0.59$, $p < 0.05$), but not BALF- or ELF-PIIINP, had a significant negative correlation with DLCO/VA. The levels of PIIINP or PICP in BALF or ELF did not correlate with FVC. Nor did the levels of S-PIIINP and S-PICP correlate with those in BALF or ELF. The concentrations of PIIINP and PICP in BALF correlated significantly with each other ($r = 0.60$, $p < 0.01$).

During the follow-up period of six years, 7/18 patients died of fibrosing alveolitis, 3 of malignancy and 1 of an unknown cause. Detectable BALF-PIIINP had no prognostic significance if all the deaths were included, but predicted a poor prognosis in fibrosing alveolitis (Table 8, Table 9.). Diffusion capacity, when assessed either in absolute or percentage values, was lower in the patients who died of pulmonary fibrosis (4.24 ± 1.3 mmol/min/kPa, 64.0 ± 8.3 %) than in those who survived (5.81 ± 2.0 mmol/min/kPa, 85.7 ± 26.3 ($p < 0.05$ Mann-Whitney U-test). The specific diffusion coefficient did not reveal any statistical differences between the two groups.

Table 8. Prognostic significance of BALF procollagen markers in fibrosing alveolitis (all causes of death) (II).

		All deaths (n=11)	Alive (n=7)	p *
BALF-PIIINP	Detectable	7 / 11	2 / 7	0.17
	Non-detectabl	4 / 11	5 / 7	
BALF-PICP	Detectable	6 / 11	3 / 7	0.50
	Non-detectable	5 / 11	4 / 7	

* Tested with Fisher's exact probability test

Table 9. Prognostic significance of BALF procollagen markers in fibrosing alveolitis (patients who died of fibrosing alveolitis) (II).

		Died of fibrosing alveolitis (n=7)	Alive (n=7)	p*
BALF-PIIINP	Detectable	6 / 7	2 / 7	0.05
	Non-detectable	1 / 7	5 / 7	
BALF-PICP	Detectable	4 / 7	3 / 7	0.50
	Non-detectable	3 / 7	4 / 7	

* Tested with Fisher's exact probability test

5.3. Lung collagens in open lung biopsies in lung sarcoidosis and fibrosing alveolitis (IV)

5.3.1. Normal lung

Normal alveolar walls and the surrounding capillaries showed procollagen I aminoterminal propeptide positivity in linear and continuous fibres. Detectable immunoreactivity for PINP was found in the middle of the interstitium of the alveolar walls (see Image 1, panel A in paper IV). The bronchiolar walls contained immunoreactivity for PINP within the connective tissue that separates the smooth muscle into bundles (see Image 1, panel C in paper IV). PINP could be detected in the tunica media layer of both arteries and veins. In visceral pleura, faint PINP immunopositivity was seen in the area between the submesothelial layer of the pleura and the alveoli of the lung.

The staining for procollagen III aminoterminal propeptide within the alveolar walls appeared to be stronger and the distribution was different from that seen in the immunoreactivity for PINP (see Image 1 in paper IV). PIIINP was localised near the epithelial cells lining alveolar walls and the cells in the interstitium, giving a spiral appearance (see

Image 1, panel B in paper IV). PIIINP was also detected around the capillaries very close to the BM. PIIINP was further expressed in the bronchioli as intensive linear fibres underneath the basement membrane (see Image 1, panel D in paper IV). Furthermore, immunoreactivity of PIIINP was seen in the tunica intima, media and adventitia layers of both arteries and veins. PIIINP was also expressed as linear fibres in the pleura underneath the BM of detached mesothelial cells. Fainter immunoreactivity was detected in the same area as PINP, i. e. between the submesothelial layer of the pleura and the alveoli of the lung. No intracellular immunoreactivity for PINP or PIIINP was seen in normal lung.

5.3.2. Sarcoidosis

Immunoreactivity for PINP was detected mainly within granulomas in sarcoidosis. It was exhibited as intracellular granular spots localising mainly in the area between the epithelioid cells and the lymphocytes (see Image 4 in paper IV). Occasionally, giant cells in granulomas expressed immunoreactivity for PINP.

PIIINP was exhibited mainly as extracellular linear and reticular fibres around the granulomas, not as intracellular spots similar to PINP (see Image 4 in paper IV). Some faint fibres were also seen in the inner parts of granulomas. The extracellular immunoreactivity for PIIINP was stronger than that for PINP around the lymphocytes outside granulomas. Intracellular immunoreactivity for PIIINP was not found in sarcoidosis.

5.3.3. Fibrosing alveolitis

Immunoreactivity for PINP was observed in areas of recent epithelial damage both intracellularly and in the extracellular space in usual interstitial pneumonia. Intracellular PINP was expressed mainly in the newly formed fibrosis, i.e. fibroblast foci, as intracellular spots within fibroblasts and myofibroblasts (see Image 2 in paper IV). The extracellular expression for PINP was strongest in the areas of ongoing epithelial regeneration with type II pneumocytes (see Images 2 and 3 in paper IV). Faint immunoreactivity of PINP could be detected extracellularly underneath the totally regenerated epithelium with type II pneumocytes or metaplastic bronchiolar-type cells (see Image 3 in paper IV). Occasionally, some immunoreactivity for PINP was also detected as short extracellular fibres. Furthermore, newly developed fibrosis within alveolar walls in areas of ongoing remodeling and in intra-alveolar and incorporating fibrosis contained remarkable positivity for PINP immunoreactivity intracellularly (see Image 2, panel C in paper IV). In some cases, linear and reticular immunopositivity for PINP was observed around alveolar macrophages (see Image 3, panel E in paper IV).

Procollagen III aminoterminal propeptide was expressed mainly as extracellular linear fibres underneath the BM of the regenerated epithelium. The immunoreactivity for PIIINP was observed in both intra-alveolar and incorporating fibrosis (see Image 2 in paper IV). Immunoreactivity for PIIINP was most intense in the areas of regenerated metaplastic alveolar epithelium or bronchiolar-type epithelium (see Image 3 in paper IV), while, unlike PINP, it was not expressed in recent epithelial damage. In these areas, faint

and slim immunopositive fibres for PIIINP were located underneath the BM. The immunoreactivity for PIIINP, though not for PINP, was exhibited marginally around lymphocytes. The expression of PIIINP was detected around clusters of intra-alveolar macrophages, as was the case with PINP (see Image 3, panels E and F in paper IV). Immunoreactivity for PIIINP was not seen intracellularly in fibrosing alveolitis.

5.4. Procollagen I carboxyterminal propeptide and procollagen III aminoterminal propeptide in bronchoalveolar lavage fluid and serum in asbestos-related lung diseases (III)

Forced expiratory volume in one second and diffusion capacity were significantly decreased in asbestosis compared with the patients without parenchymal involvement (Table 3). The numbers of asbestos bodies in the four of five patients with asbestosis without open biopsy were 2.4 AB/ml, 12.4 AB/ml, 130 AB/ml and 772 AB/ml in BALF. Twelve patients without parenchymal changes had no AB, 14 had 0-1 AB/ml and 11 had more than one AB/ml in BALF.

All of the five patients with asbestosis had significantly higher levels of PICP in BALF and ELF than the 37 patients without parenchymal involvement (Table 10, Fig. 2). In the latter group, 6 of the 25 patients with pleural plaques and two of the 12 patients without pleural involvement had detectable PICP in BALF and ELF. The levels of the procollagen markers of these two groups did not differ significantly (Fig. 2). The serum level of PICP and the levels of PIIINP in serum, BALF and ELF did not show significant differences between any of these groups (Table 10). PIIINP was detectable in BALF in one of the 5 patients with asbestosis, in three of the 25 patients with pleural involvement and in none of those without pleural involvement (Not shown). The levels of PICP or PIIINP in patients with no parenchymal involvement did not differ in relation to the amount of asbestos bodies in BALF (Fig. 3). One patient of the asbestosis group had an elevated S-ALAT value, suggesting a possible hepatic lesion. As liver diseases are known to elevate S-PIIINP, the value of this patient was excluded.

There was no statistically significant difference in the levels of PICP in BALF between the asbestosis and fibrosing alveolitis (II) patients, but the difference between the asbestosis patients and the controls (I) was highly significant ($p < 0.0001$). BALF-PICP but not BALF-PIIINP was significantly higher in the subjects exposed to asbestos fibres without parenchymal involvement than in the controls. In the sarcoidosis patients (I) the level of PIIINP in BALF was higher ($p < 0.05$) but the level of PICP in BALF lower ($p < 0.01$) than in the patients with asbestosis.

Table 10. Concentrations of PIIIINP and PICP in serum, BALF, and ELF in the patients with asbestosis and those without parenchymal involvement (III).

		Asbestosis		No parenchymal involvement	
		Mean \pm SD	Median	Mean \pm SD	Median
S-PIIINP#	$\mu\text{g/l}$	2.3 \pm 0.7	2.5##	2.8 \pm 0.8	2.7
S-PICP#	$\mu\text{g/l}$	108.6 \pm 28.2	104.5	120.4 \pm 33.7	114.0
BALF-PIIINP	$\mu\text{g/l}$	0.2 \pm 0.4	0	0.1 \pm 0.6	0
BALF-PICP	$\mu\text{g/l}$	9.8 \pm 1.8	10.8	0.6 \pm 1.3	0. ***
ELF-PIIINP	$\mu\text{g/l}$	11.6 \pm 25.9	0	5.4 [§] \pm 33.8	0
ELF-PICP	$\mu\text{g/l}$	488.9 \pm 208.8	423.9	22.6 [§] \pm 50.6	0. ***

Available from 36 patients with no parenchymal involvement. ## One patient excluded because of elevated S-ALAT. § ELF value not available for one patient with no parenchymal involvement because of missing BALF-urea. The significance of the difference was tested with the Mann-Whitney U-test. ***: $p < 0.001$.

All the patients with exposure to asbestos fibres without parenchymal involvement were invited for a control visit after a follow-up period of 7 years. Three patients had died from causes unrelated to asbestos. Thirty (81 %) of the 37 patients responded. None of these patients had developed asbestosis when assessed with chest radiography and diffusion capacity. One patient had an infiltrate caused by a lung carcinoma, and one patient had a previously diagnosed pulmonary metastasis due to a thyroid malignancy. The pleural plaques had only progressed in one patient. None of those with normal chest radiographs had developed plaques.

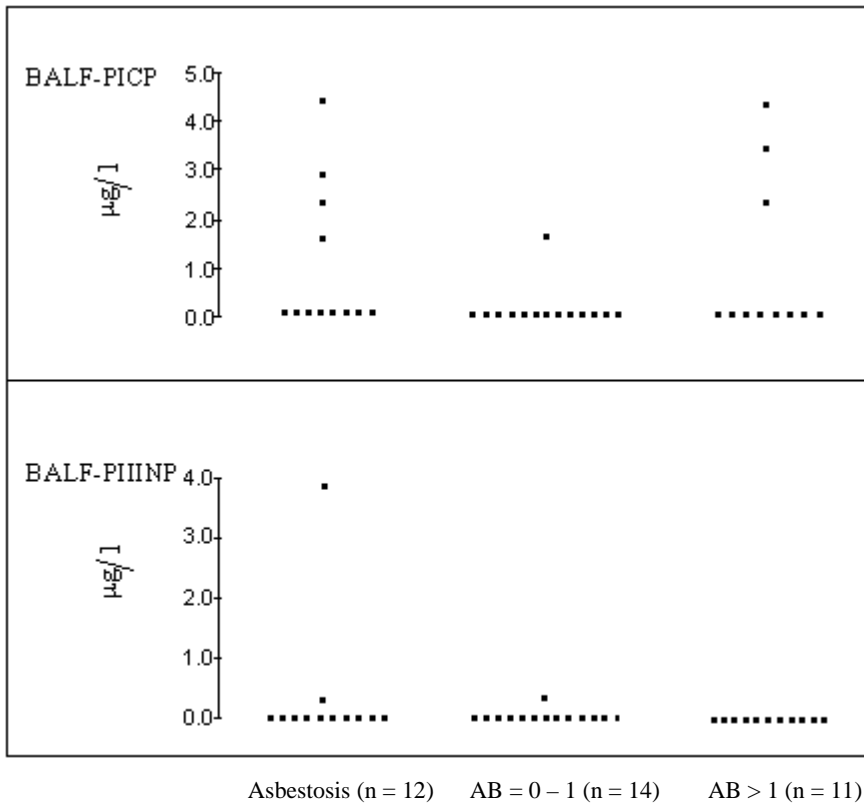


Fig 3. Individual values of PICP and PIIINP in BALF in subjects exposed to asbestos fibres without parenchymal involvement in relation to the number of asbestos bodies/ml (AB) in BALF.

6. Discussion

6.1. Study population

The study population comprised a total of 160 patients, of whom 120 underwent bronchofiberscopy and bronchoalveolar lavage because of symptoms and/or findings suggestive of interstitial lung disease or minor changes in chest radiographs subsequent to asbestos exposure. These patients constituted a clinically representative sample of patients with sarcoidosis (Pietinalho *et al.* 1995) and fibrosing alveolitis (Scott *et al.* 1990, Coultas *et al.* 1994). Five (25%) of the sarcoidosis patients with parenchymal involvement had been previously diagnosed and two of them (10%) were on oral corticosteroid medication. The BAL-procedure was performed to estimate the current activity of the disease as comparable to a new case. All of the sarcoidosis patients without parenchymal changes were newly diagnosed. None of the patients in the fibrosing alveolitis group or with asbestos exposure had had prior medication for pulmonary disease. The sarcoidosis patients were younger and the fibrosing alveolitis patients older than the controls in this study, which is in line with the fact that sarcoidosis is more common in younger and fibrosing alveolitis in older populations (Coultas *et al.* 1994, Pietinalho *et al.* 1995). The mean age of the asbestosis patients was 55 years and that of the asbestos exposure patients 54 years. This is in agreement with the latency of 15-20 years before asbestosis or pleural changes develop after the beginning of exposure (Becklake 1976). The Ethics Committee of Päivärinne Hospital recommended avoidance of any additional procedures not necessary for the diagnosis or treatment of the patients. Because of this, the control group comprised 17 patients who had been examined because of minor respiratory symptoms, and there were no healthy controls.

The BALF and serum levels of procollagen I carboxyterminal and III aminoterminal propeptides were analysed from these 120 patients and 17 controls. At the time of the serum and BALF analyses, no method for procollagen I aminoterminal propeptide was available.

A sample of 23 patients were obtained from the pathology files of the Department of Pathology, University of Oulu, Finland. The files were searched for typical histopathological diagnoses of sarcoidosis or usual interstitial pneumonia. The clinical data were obtained from the case histories. The open lung biopsies were stained immunohisto-

chemically for procollagen I and III aminoterminal propeptides. Antibodies for procollagen I carboxyterminal propeptide were not available at the time of the immunohistochemical study.

6.2. Detection of procollagens in serum, BALF and ELF

Procollagens are produced intracellularly and secreted to extracellular space, where procollagen propeptides are then cleaved from procollagen molecules by specific proteases (Prockop *et al* 1979). Cleavage of carboxyterminal propeptide is essential for the formation of collagen fibres (Miyahara *et al.* 1982), but type III collagen retains commonly part of the aminoterminal propeptide in the collagen molecule, forming pN-collagen, which has been detected in mature collagen fibres (Karttunen *et al.* 1989). When these fibrils are later degraded, the antigenic material is liberated into the extracellular space (Risteli & Risteli 1986). As known, commercial radioimmunoassay kits for PIIINP employing bovine antigen measure partly type III pN-collagen as collagen fibrils degrade (Risteli & Risteli 1990). All except one of the previous studies have used commercial radioimmunoassay kits from two manufacturers, employing bovine antigen for the analysis of PIIINP in BALF and in serum. In the present study, human antibodies were used for the assays. The information of BALF-PICP in sarcoidosis, fibrosing alveolitis or asbestos-exposed patients is limited to one preliminary study without further information of the methodology (Tukiainen *et al.* 1994).

In the present study, the original serum methods for analysing PIIINP and PICP were modified to better detect the low propeptide concentrations in BALF, and the concentrations were also calculated in ELF as estimated by the urea method (Rennard *et al.* 1986) for the first time. Commercial kits used for serum samples are usually adjusted to measure levels within and above the normal range, and their reliability may diminish at concentrations close to the detection limit. An essential problem in investigating soluble constituents in BALF is the question of the detection limit because of the highly diluted samples. Some of the previous publications have used various methods to concentrate BALF (table 1). Most studies have used ultrafiltration. The concentration factor may vary even in the same study between 10- and 100- fold (Low *et al.* 1992). In some studies (Low *et al.* 1983, Bjermer *et al.* 1986, O'Connor *et al.* 1989, Low *et al.* 1992, Schaberg *et al.* 1994), the concentration of PIIINP has been adjusted to the albumin or protein concentration, which also affects the comparability of the previous studies. The only study using the same commercial radioimmunoassay method and employing the same human antigens as the present study did not report any difference in the levels of PIIINP in BALF or serum between sarcoidosis patients and controls. The BALF analyses were made with the kit for serum without any pre-assay processing of BALF. (Milman *et al.* 1995).

Some further reasons why the previous studies on procollagen propeptides in serum and BALF have been so contradictory may include the clinical limitations of the utility of BALF itself (Davis 1994) and the fact that there has been a variety of methods for analysing procollagen peptides in BALF and serum as well as the pre-assay concentration of BALF. There is a large range of normal values for the soluble constituents and differential counting of cell populations in BALF, depending on the method used or, for example, the

smoking habits. Thus, abnormalities can rarely be considered specific. Airspace cells and secretions may not reflect interstitial processes, and the composition of epithelial lining fluid may change during bronchoalveolar lavage. (Davis 1994)

Some of the contradictory results of many previous studies can be explained partly with the above restrictions of the BALF assay and the technical variation of processing BALF as well as with the variable methods used to detect procollagen peptides. Based on the above considerations, some of the methodological problems have been avoided in the present study.

6.3. Accumulation and distribution of type I procollagen and pN-collagen in sarcoidosis and fibrosing alveolitis as detected in immunohistochemical evaluation of open lung biopsy specimens (IV)

Small propeptide molecules disappear during the processing of histological specimens. Intracellularly, aminoterminal propeptides are attached to the procollagen molecules, and the antibodies to procollagen peptides obviously react with these. In the extracellular matrix, however, the aminoterminal domains still exist in pN-collagens as part of collagen fibres (Karttunen *et al* 1989, Risteli & Risteli 1990) and can be detected with antibodies to aminoterminal propeptides. The immunoreactivity of PINP detects intracellularly type I procollagen and extracellularly N-terminal extension of type I pN-collagen molecules in the collagen molecule, whereas PIIINP detects type III procollagen and type III pN-collagen correspondingly.

A notable portion of the previous data concerning the histological findings on collagens in lungs in interstitial disease are old and only pertain to mature collagens. No previous histochemical data are available on immunoreactivity for PIIINP and PINP in normal human lung. In the present study, type I pN-collagen was localised mainly in the middle of the interstitium of the alveolar wall, while type III pN-collagen was observed near the alveolar epithelium cells beneath the basement membrane. This suggests that type I pN-collagen may have a connection with the other extracellular matrix proteins of the interstitium, whereas type III pN-collagen may be associated with BM proteins, such as collagen IV and laminin. No sign of intracellular procollagens suggesting active production of collagens was detected, which is in line with the previous findings on the absence of immunoreactivity for PICP in normal human lung (McDonald *et al.* 1986, Roman *et al.* 1995). It is known that collagen I is localised in alveolar septa, whereas collagen III is localised in capillary walls and as aggregates of collagen in normal lung (Madri & Furthmayr 1980). Some collagen I has also been detected in alveolar walls, and especially, in pleural, peribronchial and perivascular connective tissue of normal lung (Bateman *et al.* 1981).

In sarcoidosis, the present study shows that procollagen type I was expressed intracellularly within spindle-shaped cells. It is not known if some of the fibroblasts of sarcoid granulomas could eventually be myofibroblasts. In some cases, type I procollagen was occasionally observed in multinucleated giant cells of sarcoid granulomas, suggesting that macrophages or cells derived from monocytes may produce type I procollagen in sarcoidosis. Taken together, the present findings on elevated PINP and PIIINP in ELF in sarcoi-

dosis (I) suggest that alveolar macrophages may be able to synthesise type I and III collagens. The expression of type III pN-collagen was more prominent around lymphocytes than that of type pN-collagen around the granuloma. Type III procollagen was not observed intracellularly in sarcoidosis. Both collagens I and III have been found previously in sarcoid granulomas (Bateman *et al.* 1981, Peyrol *et al.* 1986). In the early stages they are distributed only peripherally but in the mature stages they can be found both around and within granulomas (Peyrol *et al.* 1986). Previously, immunoreactivity for PICP has been shown to concentrate in cells in the granulomas, where it was distributed within multinucleated giant cells, macrophages and surrounding fibroblasts and in fibroblasts scattered throughout the collagen fibrils between the granulomas. (Roman *et al.* 1995).

In the present study, the most prominent immunohistochemical accumulation of both procollagens was localised in the areas of alveolar epithelial damage, newly formed fibrosis and epithelial cell regeneration in fibrosing alveolitis. In the temporal process of fibrosing alveolitis, however, the expressions of type I pN-collagen and type III pN-collagen were very different. Type I pN-collagen was expressed mostly in the areas of damaged or deficient alveolar epithelium, while type III pN-collagen accumulated mainly at the newly formed fibrosis underneath the regenerative alveolar or bronchiolar-type epithelium. It is possible that the presence of type I pN-collagen may be important during epithelial damage, while type III pN-collagen operates mainly in the local healing process. Type III pN-collagen immunoreactivity was usually more abundant than the immunoreactivity of type I pN-collagen. The immunoreactivity for type III pN-collagen was strongest underneath the BM of regenerating metaplastic alveolar or bronchiolar type epithelium. Type III pN-collagen immunoreactivity was also present in the areas of denser fibrosis, and not only in the loose, newly formed fibromyxoid lesions. This suggests that immunoreactivity for type III pN-collagen rather than that for type I pN-collagen is present in areas of old fibrosis. Old fibrosis in fibrotic lung diseases has been shown to mainly compose of type I collagen (Bateman *et al.* 1981). It has to be emphasised that the type I procollagens and type I and III pN-collagens in the present study reflect mainly the active synthesis of collagen types I and III rather than the total amount of mature type I and III collagen. Collagen III is known to be located at subepithelial sites or in loosely arranged fibrils between densely packed type I collagen fibres in alveolar walls (Bateman *et al.* 1983, Takiya *et al.* 1983). Interstitial and alveolar fibroblasts stained intensely with PICP in active fibrosis, but not in clinically quiescent disease (McDonald *et al.* 1986). Not all of the patients with UIP had collagen-synthesising fibroblasts, and PICP-positive fibroblasts showed highly variable staining with PICP. Stained fibroblasts made up subepithelial clusters, so called fibroblast foci, near the air-tissue interface, and the cells were large and intensively stained. (Kuhn *et al.* 1989, Kuhn & McDonald 1991). Immunoreactivity of PINP has been detected in myofibroblasts in fibroblast foci in fibrosing alveolitis (Bensadoun *et al.* 1996). In the present study, immunoreactivity for type I procollagen was typically observed as intracellular spots within the clusters of fibroblasts and myofibroblasts in the fibroblast foci. These results are also in agreement with the previous findings of immunoreactivity of PICP (Kuhn *et al.* 1989).

6.4. PIIINP and PICP in serum, BALF and ELF in patients with sarcoidosis, fibrosing alveolitis and asbestos-related lung diseases (I-III)

The previous reports concerning the levels of serum and BALF-PIIINP in sarcoidosis, fibrosing alveolitis and asbestosis have been contradictory. Several studies have indicated that PIIINP is elevated in BALF of sarcoidosis patients (Low *et al.* 1983, Bjermer *et al.* 1986, Bjermer *et al.* 1987B, Bjermer *et al.* 1991, Schaberg *et al.* 1994, Straub *et al.* 1995, Tukiainen *et al.* 1994), although some studies have failed to reveal any difference between controls and sarcoidosis patients (Cantin *et al.* 1988, Milman *et al.* 1995). Serum levels of PIIINP have been found to be elevated compared with controls (Bacchella *et al.* 1996, Luisetti *et al.* 1990, Schoenfeld *et al.* 1996), but again, there is a study where no elevation was detected (Milman *et al.* 1995).

The elevated PIIINP in BALF in fibrosing alveolitis patients compared to controls is more like a rule than an exception (Harrison *et al.* 1993, Low *et al.* 1983, Low *et al.* 1992, Cantin *et al.* 1988, Bjermer *et al.* 1989, Kuroki *et al.* 1995, Fujimoto *et al.* 1995, Tukiainen *et al.* 1994). There is evidence of elevated levels of serum PIIINP (Low *et al.* 1983, Low *et al.* 1992, Harrison *et al.* 1993), but contradictory findings have also been reported (Watanabe *et al.* 1985). Information on BALF-PICP is limited to a study which showed elevated levels of BALF-PICP in sarcoidosis and fibrosing alveolitis compared to controls (Tukiainen *et al.* 1994).

Knowledge of the propeptide levels in human asbestos-related diseases is limited, most studies being experimental. According to the only study on BALF-PIIINP in asbestos-related diseases, the level of PIIINP in BALF was observed to be elevated in asbestosis compared to asbestos-exposed subjects without parenchymal disease (Begin *et al.* 1986). PICP but not PIIINP was elevated in BALF in asbestos-exposed subjects (Tukiainen *et al.* 1994). In addition, one study suggests that serum PIIINP may be a useful index of asbestosis (Cavalleri *et al.* 1988). There is also evidence of the elevation of PIIINP in BALF in progressive silicosis (Begin *et al.* 1987a).

In the present study, serum PIIINP was slightly elevated in 13% of the patients with sarcoidosis and 22% of the patients with fibrosing alveolitis, but not in asbestosis or asbestos-exposed subjects without parenchymal involvement. BALF and serum PIIINP had no reciprocal correlation. Based on these considerations, it is obvious that S-PIIINP cannot be used as a marker of local fibrogenesis in lungs. The levels of serum PICP were similar in sarcoidosis, fibrosing alveolitis, asbestos-exposed subjects, asbestosis and controls. This is in line with the fact that the majority of PICP is derived from bone (Melkko *et al.* 1990). It therefore seems evident that S-PICP is a poor indicator of any lung disease.

The level of BALF-PIIINP was highest in sarcoidosis and second highest in fibrosing alveolitis, but hardly detectable in the other groups. When evaluated in the epithelial lining fluid estimated by the urea method, PIIINP was elevated in sarcoidosis (121-fold) and fibrosing alveolitis (13-fold) compared to serum, suggesting active local synthesis or possibly degradation of type III collagen in the lower respiratory tract. Procollagen I carboxyterminal propeptide in BALF was high in fibrosing alveolitis and asbestosis and was also elevated to a lesser extent in sarcoidosis, although the levels did not differ significantly. Detectable BALF-PICP was observed in some of the asbestos-exposed patients

without parenchymal involvement but not in the controls. Again, the average concentrations of PICP were higher in ELF than in serum, being twofold in sarcoidosis and fourfold in fibrosing alveolitis and asbestosis, which can be considered a sign of active synthesis of type I collagen in lungs. BALF- and ELF-PICP were elevated in the patients who may have slowly progressive development of lung fibrosis, such as asbestosis or idiopathic fibrosis, and, to a lesser extent, in sarcoidosis. On the contrary, BALF-PIIINP was highest in parenchymal sarcoidosis with an obvious local inflammatory process, whereas the level of PIIINP in BALF was relatively low in fibrosing alveolitis and hardly detectable in asbestos-exposed patients and controls. This may suggest that BALF-PICP would be a better marker of local fibrogenesis than BALF-PIIINP.

One possible explanation for the contradictory results of the previous studies other than the methodological point of view discussed above is the heterogeneity in the status and course of the diseases investigated. At the time of the diagnosis, the patients can be in various stages of the disease. We would need large numbers of patients at each stage of the diseases, i.e. large national or international studies, to be able to make further conclusions.

6.5. PIIINP and PICP as possible markers of disease activity and prognosis in sarcoidosis, fibrosing alveolitis and asbestos-related lung diseases (I-III)

The activity of sarcoidosis is difficult to assess, and only clinical findings, chest radiographs and pulmonary function tests have been suggested to be relevant markers, although several other markers have been widely investigated (Costabel *et al.* 1994). This is the case even with fibrosing alveolitis. There is some evidence of a correlation between serum and BALF-PIIINP with other suggested markers of disease activity in sarcoidosis and fibrosing alveolitis although the results diverge.

The present study on sarcoidosis revealed no correlation between BALF-PICP or BALF-PIIINP and pulmonary function tests or diffusion capacity or specific diffusion coefficient. BALF-PIIINP correlated well with S-ACE, S-IL-2R, BALF-albumin, and BALF-lymphocytes. BALF-PICP correlated with BALF-PIIINP. The patients with the highest counts of mast cells in their BALF also had higher PIIINP and PICP in BALF, and symptomatic patients had higher PIIINP and PICP levels in BALF than non-symptomatic ones, suggesting that BALF-PIIINP and -PICP may correlate with disease activity. In addition, the patients with parenchymal involvement in chest radiographs had significantly higher S-ACE and BALF- and ELF-PIIINP, suggesting an active form of the disease. The average level of BALF-PICP was slightly but not significantly higher in the parenchymal than the non-parenchymal sarcoidosis group. In previous studies, serum PIIINP has been shown to correlate with serum ACE (Luisetti *et al.* 1990, Schoenfeld *et al.* 1996), but not with ⁶⁷Ga uptake, BALF-lymphocytes, vital capacity or diffusion capacity (Luisetti *et al.* 1990). On the contrary, S-PIIINP has correlated with parameters of restriction estimated in terms of vital capacity and total lung capacity (Schoenfeld *et al.* 1996). The level of serum PIIINP has been higher in progressive sarcoidosis than inactive disease in serial measurements (Pohl *et al.* 1992). On the other hand, some other

studies have failed to show any correlation between S-PIIINP and sarcoidosis activity (Low *et al.* 1983, Poole *et al.* 1989, Milman *et al.* 1995). BALF-PIIINP has been observed to correlate inversely with vital capacity, forced expiratory volume and diffusion capacity, and it has been related to pulmonary radiological findings, serum ACE and mast cells but not to other cells in BALF (Bjermer *et al.* 1986, Bjermer *et al.* 1987b) as well as with sarcoidosis changes in bronchial biopsies (Bjermer *et al.* 1991). In the latter study, BALF-PIIINP also correlated with a poor clinical course. On the other hand, in a follow-up study of 12 months the initial BALF-PIIINP did not predict the severity of the disease assessed with lung function testing (O'Connor *et al.* 1989). BALF-PICP and PIIINP were associated with BALF-lymphocytes and the patients with advanced parenchymal changes had elevated levels of PICP and PIIINP in BALF (Tukiainen *et al.* 1994).

The present study, where BALF- and ELF-PICP but not BALF/ELF PIIINP had a significant inverse correlation with DLCO/VA, suggests a process affecting the air-blood barrier in the lungs in fibrosing alveolitis. Detectable BALF-PIIINP and decreased diffusion capacity predicted poor prognosis in a follow-up of six years. In previous studies on fibrosing alveolitis, serum PIIINP has been shown to correlate with disease activity estimated by clinical, radiological, and physiological scoring of disease severity (Low *et al.* 1992), although another study by the same author failed to reveal such correlation (Low *et al.* 1983). BALF-PIIINP has been suggested, together with elevated levels of eosinophils cationic protein in BALF, to discriminate between acute, progressive disease and stable disease (Fujimoto *et al.* 1995).

There are no previous data available of the role of procollagen propeptides in the prognosis of asbestos-exposed patients without pulmonary involvement at the time of diagnosis. Extreme experimental exposure with asbestos has led to an elevation of PIIINP in BALF and to the development of pulmonary fibrosis (Begin *et al.* 1987b, Begin *et al.* 1990). In the present study, despite the elevated BALF-PICP, none of the asbestos-exposed subjects developed asbestosis when assessed with chest radiography and diffusion capacity after a follow-up of seven years.

According to the present data, neither serum PIIINP or PICP seems to have any role in estimating the activity or prognosis of sarcoidosis, fibrosing alveolitis and asbestos-related diseases. BALF-PIIINP predicted disease activity shown by chest radiographs in sarcoidosis and a poor prognosis in fibrosing alveolitis, whereas elevated BALF-PICP suggested a fibrosing process.

7. Conclusions

1. Procollagen III aminoterminal propeptide is elevated in serum, bronchoalveolar lavage fluid (BALF) and epithelial lining fluid (ELF) in patients with sarcoidosis and fibrosing alveolitis compared to controls.
2. The level of procollagen III aminoterminal propeptide in BALF correlates with disease activity and is related to parenchymal changes in sarcoidosis and a poor prognosis in fibrosing alveolitis.
3. Procollagen I carboxyterminal propeptide in BALF and ELF but not in serum is elevated in patients with sarcoidosis, fibrosing alveolitis and asbestosis. Procollagen I carboxyterminal propeptide in BALF is rather a marker of local type I collagen synthesis in lungs than a marker of inflammatory activity of these diseases.
4. Immunoreactivities for procollagen type I and III propeptides are increased in fibrosing alveolitis and sarcoidosis compared to normal lung in histological lung biopsies. In fibrosing alveolitis, type I and III pN-collagens are distributed especially in areas of alveolar epithelial damage with newly formed fibrosis and epithelial regeneration. They are differently distributed and expressed in sarcoidosis and fibrosing alveolitis, suggesting specific roles for type I and III collagens in pulmonary fibrogenesis.

8. References

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