

Pleural fluid interferon- γ and tumour necrosis factor- α in tuberculous and rheumatoid pleurisy

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Pleural fluid interferon- γ and tumour necrosis factor- α in tuberculous and rheumatoid pleurisy. T. Söderblom, P. Nyberg, A.-M. Teppo, M. Klockars, H. Riska, T. Pettersson. ©ERS Journals Ltd 1996.

ABSTRACT: Tuberculous and rheumatoid pleural effusions show features suggesting a strong local cellular immune response. Pleural fluid (Pf) from patients with tuberculosis, rheumatoid arthritis (RA) and other diseases were compared with respect to interferon- γ (IFN- γ) and tumour necrosis factor- α (TNF- α).

Immunoassays were used to determine Pf-IFN- γ and Pf-TNF- α in 102 patients, including 11 with RA, 31 with verified tuberculosis, 23 with suspected tuberculosis, 11 with pneumonia, 14 with lung cancer and 12 with congestive heart failure.

Measurable Pf-IFN- γ occurred exclusively in patients with verified (median 1.8 ng·mL⁻¹; 95% confidence interval (95% CI) 0.63–4.0 ng·mL⁻¹) or suspected (0.37 ng·mL⁻¹; 95% CI 0–0.7 ng·mL⁻¹) tuberculosis. The highest median Pf-IFN- γ was observed in those patients who showed a positive pleural fluid culture for *Mycobacterium tuberculosis*. In pleural effusions due to other diseases, including RA, IFN- γ was undetectable. The highest Pf-TNF- α occurred in verified tuberculosis (median 198 ng·L⁻¹; 95% CI 169–222 ng·L⁻¹) and RA (210 ng·L⁻¹; 95% CI 147–231 ng·L⁻¹).

Pleural fluid interferon- γ is a highly useful marker for diagnosing tuberculous pleurisy. Although tuberculous and rheumatoid pleural effusions share several biochemical features, they are strikingly different with respect to interferon- γ .

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The diagnosis of tuberculous pleurisy is traditionally based on a positive pleural fluid culture for *Mycobacterium tuberculosis* and/or on the demonstration of a granulomatous inflammation with giant cells and caseation necrosis in pleural tissue specimens. However, in a considerable proportion of cases the diagnosis has to be established on clinical grounds alone [1].

Tuberculous pleural effusions typically show a predominance of T-lymphocytes [2–3], and high levels of adenosine deaminase (ADA) [4, 5] and soluble interleukin-2 receptor (sIL-2R) [6]. In rheumatoid pleural effusions, high ADA and sIL-2R levels have also been reported [6, 7]. Certain features of pleural fluid, such as extremely low glucose and complement C4 concentrations and high levels of neuron-specific enolase, however, differentiate rheumatoid pleurisy from tuberculous pleurisy [1, 8].

Several authors have reported high levels of interferon- γ (IFN- γ) in tuberculous pleural effusions [9–13]. The few observations on pleural fluid (Pf)IFN- γ in RA have suggested considerably lower values [12, 13]. High concentrations of tumour necrosis factor- α (TNF- α) in pleural fluid have been observed in several diseases, including tuberculosis [14, 15].

The aims of our study were to evaluate the diagnostic usefulness of Pf-IFN- γ and Pf-TNF- α determinations, and, in particular, to compare tuberculous and rheumatoid effusions with respect to these variables.

Patients and methods

Patients

The study population consisted of 102 patients with pleural effusion: 11 patients had rheumatoid arthritis (RA), 31 verified tuberculosis, 23 suspected tuberculosis, 11 pneumonia, 14 lung cancer and 12 congestive heart failure (CHF).

Patients with RA fulfilled the revised criteria of the American College of Rheumatology [16]. Tuberculosis was verified by a positive staining or culture for *M. tuberculosis* in pleural fluid or sputum, and/or by the demonstration of a granulomatous inflammation with caseation necrosis in a pleural biopsy specimen. All the cancer patients had a lung tumour and the pleural fluid and/or pleural biopsy specimen contained neoplastic cells. The diagnoses of pneumonia and CHF were based on the clinical course of the diseases and on a favourable response to antimicrobial and diuretic therapy, respectively.

Of the 23 patients with suspected tuberculosis, 20 presented with fever and/or cough, 2 with chest pain, and one was asymptomatic. In 9 patients, chest radiography showed a lung infiltrate. The skin reaction to tuberculin was positive in 21 patients (a diameter of more than 10 mm in 18 cases) and negative in two. Nineteen patients had more than 90 percent lymphocytes on a pleural fluid

differential cell count. Six patients had a known exposure to *M. tuberculosis*, three had previously received antituberculous medication because of pulmonary infiltrates, and two had a history of pleural effusion of unknown origin. One patient concurrently had bacteriologically verified cervical lymph node tuberculosis. All 23 patients responded positively to antituberculous drugs and were followed-up for at least 1 yr.

Methods

Pleural fluid samples were obtained by intercostal needle aspiration. All pleural fluids were cultured and stained for the presence of bacteria and analysed cytologically for the presence of tumour cells. Total and differential white cell counts and protein, glucose, lactate dehydrogenase and complement C3 and C4 concentrations were determined in all pleural fluids.

The pleural fluid samples were stored at -20°C until assayed for IFN- γ and TNF- α . Pf-IFN- γ from 100-fold diluted pleural fluid was determined with an enzyme-linked immunosorbent assay (ELISA) (Bender MedSystems, Vienna, Austria). According to the manufacturer, the lower detection limit of the test was 1.5 pg·mL⁻¹ and the range of variation in normal human serum <1.5–168 pg·mL⁻¹.

To exclude the possibility that rheumatoid factor present in pleural effusions interfered with the IFN- γ ELISA, pleural fluid samples from the 11 patients with RA were treated with dithiothreitol to remove rheumatoid factor.

A solid phase double antibody radioimmunoassay [17] was used to measure Pf-TNF- α . The lower detection limit of the assay was 7 ng·L⁻¹. The upper limit of normal human serum concentrations of TNF- α , defined as the mean +2SD observed in 50 healthy persons, was 40 ng·L⁻¹.

ADA activity was measured according to GIUSTI [18]. Since the activity of ADA tends to decrease during storage, we included only those ADA values which were obtained on fresh samples. Pleural biopsies were taken either under local anaesthesia with the Abrams' needle, or at thoracoscopy or thoracotomy.

Statistics

Statistical calculations were performed with Mann-Whitney's test and Spearman's correlation test.

Results

Patients with verified or suspected tuberculosis were distinguished by detectable quantities of IFN- γ in pleural fluid (median 1.8 and 0.37 ng·mL⁻¹, respectively; 95% confidence interval (95% CI) 0.63–4.0 and 0–0.7 ng·mL⁻¹, respectively). IFN- γ was undetectable in all nontuberculous effusions, including the 11 rheumatoid effusions. The highest median Pf-IFN- γ (4.0 ng·mL⁻¹) was observed in patients with a positive culture for *M. tuberculosis* in pleural fluid (fig. 1). Patients in whom the diagnosis of tuberculosis relied on sputum culture or on histological examination had somewhat lower median Pf-IFN- γ (0.78 and 0.72 ng·mL⁻¹, respectively). The median Pf-IFN- γ was still lower in patients with suspected tuberculosis (0.37 ng·mL⁻¹), but two high values occurred in this group. Two out of 31 patients with verified tuberculosis and 9 out

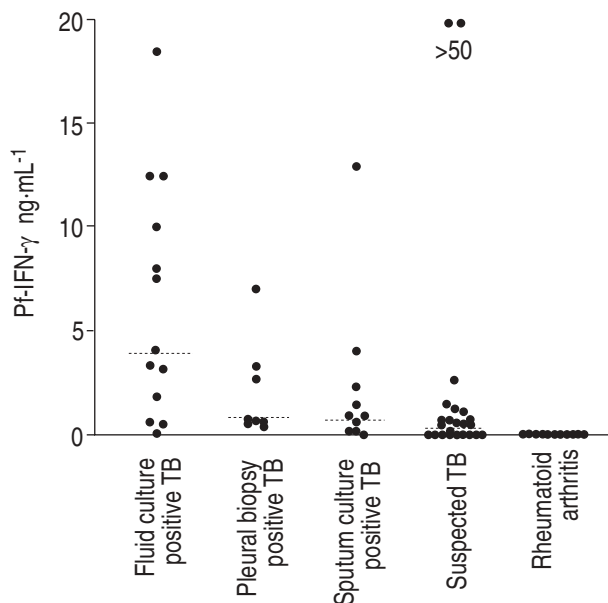


Fig. 1. – Concentration of interferon- γ (IFN- γ) in pleural fluid (Pf) of 31 patients with verified tuberculosis (TB), 23 patients with suspected tuberculosis and 11 patients with rheumatoid arthritis. Horizontal dash lines indicate median values in each group.

of 23 with suspected tuberculosis had undetectable Pf-IFN- γ . The sensitivity of detectable Pf-IFN- γ for verified tuberculosis was 94%.

After removal of rheumatoid factor by dithiothreitol treatment, IFN- γ was still below the detection limit in all 11 rheumatoid pleural effusions. The IFN- γ concentrations in pleural fluid samples of patients with tuberculous pleurisy were as high before as after dithiothreitol treatment.

The highest median Pf-TNF- α was seen in RA (210 ng·L⁻¹). Median Pf-TNF- α was significantly higher in patients with RA than in those with lung cancer (165 ng·L⁻¹; p<0.05), pneumonia (160 ng·L⁻¹; p<0.05) or CHF (121 ng·L⁻¹; p<0.005), but there was considerable overlap between all groups (fig. 2). Patients with verified or suspected tuberculosis had as high median Pf-TNF- α

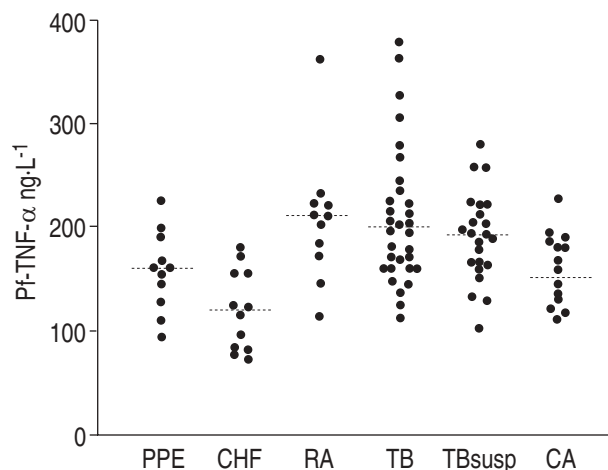


Fig. 2. – Concentration of tumour necrosis factor- α (TNF- α) in pleural fluid (Pf) of 102 patients with pleural effusions of various causes. PPE: parapneumonic effusion; CHF: congestive heart failure; RA: rheumatoid arthritis; TB: verified tuberculosis; TBsusp: suspected tuberculosis; CA: cancer. Horizontal dash lines indicate median values in each group.

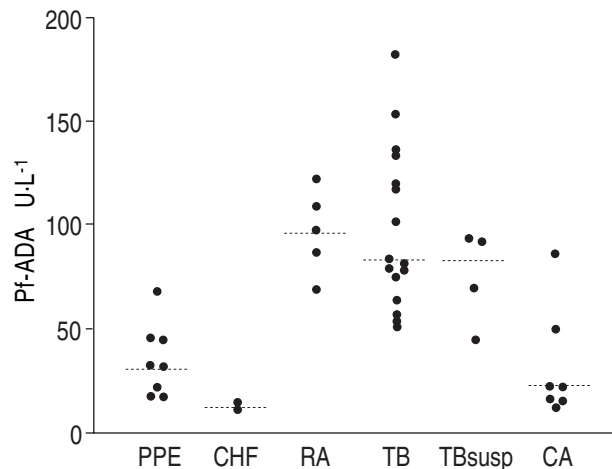


Fig. 3. – Activity of adenosine deaminase (ADA) in pleural fluid (Pf) of 42 patients with pleural effusions of various causes. Horizontal dash lines indicate median values in each group. For definitions see legend to figure 2.

(198 and 191 ng·L⁻¹, respectively) as patients with RA. Pf-IFN- γ correlated positively with Pf-TNF- α ($r=0.37$).

Although the highest median pleural fluid ADA activity (96 U·L⁻¹) was observed in RA, the highest single values were measured in patients with verified tuberculosis (fig. 3). The median Pf-ADA was significantly higher in RA than in pneumonia (32 U·L⁻¹; $p<0.005$) and cancer (21 U·L⁻¹; $p<0.01$), but was similar in RA as in verified and suspected tuberculosis (81 U·L⁻¹, each). Pf-ADA correlated positively with Pf-TNF- α ($r=0.41$). Neither Pf-IFN- γ nor Pf-TNF- α correlated significantly with pleural fluid white cell counts or lymphocyte counts.

Discussion

The main new observation in this study was that determination of Pf-IFN- γ distinguishes tuberculous effusions not only from parapneumonic and neoplastic effusions [9, 12, 13], but also from rheumatoid effusions. This difference between tuberculous and rheumatoid pleurisy was unexpected, taking into consideration the several similarities in the biochemical features of pleural fluid (e.g. high ADA levels) in these two granulomatous disorders. IFN- γ is predominantly produced by T-helper type 1 lymphocytes. Thus, the abundance of IFN- γ in tuberculous pleural effusions but not in rheumatoid effusions suggests fundamental differences between the local T-cell responses in tuberculosis and RA.

Lymphocytes from tuberculous pleural effusions produce IFN- γ when stimulated with tuberculin [2, 19, 20], or with mycobacterial cell wall proteins [14]. IFN- γ activated murine bone marrow macrophages have been shown to inhibit the *in vitro* production of mycobacterial ribonucleic acid (RNA) and to reduce the number of viable mycobacteria [21]. IFN- γ is reported to stimulate the killing capacity of macrophages through an enhanced secretion of reactive oxygen metabolites [22], and an increased production of TNF- α [23, 24]. TNF- α has been shown to induce the production of IFN- γ , possibly through a positive feedback during an ongoing immune response [25]. TNF- α is required for granuloma formation and for bacillary elimination at the site of inflammation by increasing the intracellular killing capacity of macrophages [24,

26]. The fact that tuberculous pleural effusions generally resolve without therapy suggests that the local immune response against mycobacteria is successful. In our study, there was a positive correlation between the mycobacterial burden in the pleural fluid and the local immune response as measured by IFN- γ .

In RA, the pattern of low IFN- γ and high TNF- α has also been reported to occur in synovial fluid [27, 28]. In the pathogenesis of RA, TNF- α is believed to possess a pivotal role as a proinflammatory cytokine, which induces the production of interleukin (IL)-1 and IL-6 [29]. The observation that IFN- γ levels are low in rheumatoid synovial fluid despite the high number of T-cells in the rheumatoid synovial membrane suggests that T-cells in RA are in some way downregulated [28]. This downregulation has been ascribed to an increased local production of immunoregulatory cytokines, such as IL-10 and transforming growth factor- β [30]. The results of the present study suggest that in RA similar pathogenetic events are important in both synovitis and pleurisy.

In conclusion, the abundance of interferon- γ in tuberculous pleural effusions probably reflects a strong local cellular immune response. In rheumatoid pleurisy, despite histological resemblance with tuberculous pleurisy the production of interferon- γ seems to be downregulated.

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