

Changes in serum neopterin and serum β_2 -microglobulin in subjects with lung infections

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ABSTRACT: Our aim was to investigate whether serum neopterin and β_2 -microglobulin have any value in the distinction between *Pneumocystis carinii* pneumonia (PCP) and pneumonia due to extracellular bacteria. Also, to study whether neopterin and β_2 -microglobulin would correlate with the clinical course of lung infections in human immunodeficiency virus (HIV)-positive and HIV-negative patients.

Thirty HIV-positive subjects with PCP, 9 HIV-positive patients with bacterial pneumonia, and 16 HIV-negative patients with bacterial pneumonia were investigated. Thirty eight asymptomatic HIV-positive subjects and 48 healthy blood donors were used as controls.

The HIV-positive patients with PCP and the HIV-positive subjects with bacterial pneumonia had significantly and similarly elevated levels of neopterin and β_2 -microglobulin in the acute stage. In the weeks before the acute stage of PCP, neopterin and β_2 -microglobulin had been increasing. After start of treatment, serum neopterin declined significantly, whilst serum β_2 -microglobulin remained elevated. The HIV-negative patients with bacterial pneumonia had significantly increased serum concentrations of both markers in the acute stage, and had decreasing serum concentrations in the weeks after treatment.

We conclude that neither neopterin nor β_2 -microglobulin seem to be of value in distinction between PCP and bacterial pneumonia in HIV-positive subjects. In the HIV-positive patients, neopterin may correlate partly with the clinical activity of PCP, whilst serum β_2 -microglobulin may remain elevated after PCP, despite treatment and recovery. The elevated level may, in part, be due to repeated infections and progression to acquired immune deficiency syndrome (AIDS). In the HIV-negative patients with pneumonia both parameters seem to correlate with disease activity and recovery.

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Neopterin and β_2 -microglobulin are biochemical markers for "*in vivo*" activation of cell-mediated immunity. Neopterin is a low molecular weight pteridine compound converted from guanosine triphosphate (GTP). It is released by activated human monocytes/macrophages upon stimulation with interferon-gamma produced by activated T-lymphocytes. Neopterin is a stable molecule, and is cleared from the circulation by the kidneys [1]. Beta₂-microglobulin is a membrane protein of low molecular weight. It constitutes the small constant component of the class I major histocompatibility complex. Upon immune system activation, the cells actively release β_2 -microglobulin into the circulation, and it is eliminated by glomerular filtration [2]. Raised serum neopterin and β_2 -microglobulin concentrations are found in various malignant, autoimmune and infectious diseases [3, 4]. Among the infectious agents, viruses in particular evoke cellular immune responses with high levels of neopterin and β_2 -microglobulin [5–7]. Neopterin concentration usually correlates with the clinical course. The serum concentration

is high early in the infection, and it decreases as soon as antibodies against the pathogen become detectable [6]. Serum β_2 -microglobulin is characterized by elevated concentration in the acute phase and with a slow normalization of the level in the subsequent weeks [7]. In human immunodeficiency virus (HIV) infection, the two serological markers are continuously elevated, and increasing concentration is associated with progression to acquired immune deficiency syndrome (AIDS) [8].

The present study was undertaken in order to estimate serum neopterin and β_2 -microglobulin levels in HIV-positive patients with pneumonia caused by *Pneumocystis carinii* and bacteria. We wanted to investigate whether measurements of the two markers could distinguish between the two types of pneumonia in HIV-positive patients. In addition, we wanted to study whether both markers would correlate with the clinical course of lung infections in HIV-positive and HIV-negative subjects. We expected that prolonged infection with *Pneumocystis carinii* in lung alveolae would stimulate the activation

of the cellular immune system more intensively than acute infection with encapsulated extracellular bacteria. These bacteria are not usually connected with activated cellular immunity. The virulence of pneumococci and *Haemophilus influenzae* may be related to the encapsulation and inadequate levels of specific antibodies [9]. The host defences against *Pneumocystis carinii* may involve both humoral and cellular immune factors. Interferon-gamma from activated T-cells and tumour necrosis factor from activated macrophages may contribute to the host defence against *Pneumocystis carinii* [10]. As patients with AIDS may have high levels of interferon-gamma [11], and tumour necrosis factor [12], in the blood we anticipated that patients with *Pneumocystis carinii* pneumonia might have high levels of neopterin and β_2 -microglobulin.

Material and methods

A total of 250 sera from 145 subjects was investigated for neopterin and β_2 -microglobulin concentrations; 1–5 serum samples from each patient. All HIV-positive patients were regularly examined at the out-patient clinic of the Department of Infectious Diseases, Morselisborg Hospital, Aarhus, Denmark. The HIV-positive and HIV-negative patients with pulmonary symptoms were admitted to the ward and examined clinically, and blood gas analysis and chest X-ray were performed. If an infiltrate was demonstrated, transtracheal aspiration or bronchoalveolar lavage were performed in order to obtain material for microbiological examinations. All specimens obtained by bronchoalveolar lavage and transtracheal aspiration were examined for aerobic and anaerobic bacteria, fungi, *Legionella pneumophila* and mycobacteria by culture and microscopy. The specimens were also examined for *Pneumocystis carinii* by direct microscopy after Papanicolaou staining and Gomori methamine staining. The aetiological diagnosis of bacterial pneumonia was based on the bacterial organisms in bronchial secretions obtained by transtracheal aspiration. Transtracheal aspiration was performed by percutaneous puncture below the thyroid cartilage; material obtained from the bronchi by this method is generally considered to be sterile, except in patients with chronic bronchitis where colonization may occur. For this reason, we have excluded patients with chronic bronchitis. We have not attempted to perform quantitative microbiological investigations.

The subjects investigated were divided into the following groups:

Group 1

Thirty AIDS patients (27 males and 3 females) with *Pneumocystis carinii* pneumonia (PCP), with a mean age of 40 yrs (range 18–71 yrs). In 23 patients, PCP was the first presenting manifestation of AIDS. Mean CD4-cell count was $96 \pm 100(\text{SD}) \times 10^6 \cdot l^{-1}$. Seven patients had both PCP and another AIDS-defining disease. Three of these patients had Kaposi's sarcoma, two patients had oesophageal candidiasis, and two had toxoplasmosis of

the central nervous system. Their mean CD4-cell count was $23 \pm 15 \times 10^6 \cdot l^{-1}$.

The suspicion of PCP was based on clinical features, with fever, increasing dyspnoea and cough, hypoxaemia and abnormal chest radiographs. PCP was diagnosed definitively by demonstrating the organisms in bronchial secretions obtained by bronchial lavage and in a few cases by transtracheal aspiration. The duration of illness had been 1–12 weeks (mean 4 weeks) before admission to the ward. Seventy eight percent of the patients presented with fever, 82% had nonproductive cough and dyspnoea at rest or upon exertion, 26% had a severe weight loss, and 45% had systemic symptoms of malaise and fatigue. Eighteen of the patients had hypoxaemia, and chest X-rays from 27 of the PCP patients showed bilateral interstitial infiltrates. Two patients had a lobar infiltrate, and one had a normal chest X-ray. In 26 of the patients, the *Pneumocystis carinii* was demonstrated in samples obtained by bronchoalveolar lavage, and in four patients in transtracheal aspirate. In two of the PCP patients *Haemophilus influenzae* was also demonstrated. In none of the patients could mycobacteria, fungi or anaerobic organisms be demonstrated by culture. None of the smears showed mycobacteria or fungi.

All 30 patients were investigated for neopterin and β_2 -microglobulin concentrations at the acute stage. Sera were available for investigation in 10 patients prior to PCP and in 27 patients in the postinfectious period.

Group 2

Nine HIV-positive patients (6 males and 3 females) with acute bacterial pneumonia. Six had pneumococcal pneumonia, and three had mixed infections with pneumococci and *Haemophilus influenzae*. The mean age was 39 yrs (range 26–65 yrs). Four of these patients had AIDS, diagnosed on the basis of previous PCP (1 patient), B-lymphoma (2 patients), and oesophageal candidiasis (1 patient). The other five had HIV-related symptoms. The mean CD4-cell count was $72 \pm 68 \times 10^6 \cdot l^{-1}$.

The diagnosis of bacterial pneumonia was based on chest radiographs, clinical features, and demonstration of bacteria in bronchial secretions obtained by transtracheal aspiration. The patients had had fever and productive cough lasting from a few days until one week before admission to the ward. Seven patients had a lobar infiltrate, and one had bilateral infiltrates. Only one patient had a very slight infiltrate, but because of clinical symptoms with fever and cough, mild hypoxaemia and a positive microscopy showing pneumococci, the patient was included in this group. The blood gas analysis showed only a mild hypoxaemia in three patients. Culture and/or microscopy of material obtained by transtracheal aspiration revealed pneumococci in six cases, and both pneumococci and *H. influenzae* in three cases. None of the cultures showed mycobacteria, fungi or anaerobic bacteria. In none of the smears could mycobacteria or fungi be demonstrated, and PCP was not demonstrated by microscopy. All patients recovered after treatment with beta-lactam antibiotics.

Group 3

Thirty eight asymptomatic HIV-positive patients (33 males and 5 females) with a mean age of 35 yrs (range 20–55 yrs). The mean CD4-cell count was $843 \pm 315 \times 10^6 \cdot l^{-1}$.

Group 4

Sixteen HIV-negative patients (9 males and 7 females) with acute community-acquired pneumonia. Twelve had pneumococcal infections, four had mixed infections with pneumococci and *H. influenzae*. The mean age was 53 yrs (range 26–87 yrs). The diagnosis was based on chest radiographs, clinical features, and demonstration of bacteria in bronchial secretions obtained by transtracheal aspiration. The patients had respiratory symptoms, with fever, productive cough and chest pain for 1–14 days (mean 4 days) before admission to the ward. Chest X-rays showed a lobar infiltrate in 14 and bilateral segmental infiltrations in two. In eight of the patients with pneumococcal infection the bacteria were demonstrated by culture, and in four patients only by smear. The mixed infections in four patients were based on culture. None of the cultures showed mycobacteria, fungi or anaerobic organisms. No mycobacteria or fungi were demonstrated in smears.

All 16 patients were investigated at the acute stage. Sera from 10 of the patients were available for investigation in the postinfectious period.

Group 5

Forty eight HIV-negative, healthy blood donors (27 males and 21 females) from the Blood Bank, Aarhus University Hospital, Skejby, Denmark. The mean age was 41 yrs (range 21–64 years).

All the participants in the study population had normal serum creatinine, and none of the HIV-negative subjects had any chronic or other intercurrent diseases. All the sera were stored at -20°C until used.

The subjects gave written consent to participate in the study, which was performed according to the declaration of Helsinki.

Methods

Neopterin and β_2 -microglobulin levels in serum were quantitated by using commercial kits. Neopterin was quantitated by using a radioimmunoassay kit. β_2 -microglobulin was assessed by using a solid phase time-resolved fluoroimmunoassay kit, based on competition between europium-labelled β_2 -microglobulin and sample β_2 -microglobulin for monoclonal anti- β_2 -microglobulin antibodies [13]. The methods used for measuring neopterin and β_2 -microglobulin were performed according to instruction manuals of the manufacturers (Neopterin-RIAcide, Henning, Berlin, GMBH, Germany, and Pharmacia DELFIA System, Uppsala, Sweden). In this study, the neopterin kit had intraassay and interassay variations of

8 and 9%, respectively, and in the DELFIA β_2 -microglobulin assay the intraassay and interassay variations were 7 and 11%, respectively.

Statistical analysis

The results were expressed as mean values and compared by using the Wilcoxon and the Mann-Whitney test. The level of significance was established at $p < 0.05$.

Results

Neopterin (table 1 and fig. 1)

Serum neopterin concentration in HIV-negative blood donors (Group 5) was significantly lower ($p < 0.001$) than in asymptomatic HIV-positive subjects (Group 3) (table 1).

In HIV-negative patients with acute bacterial pneumonia due to pneumococci and *H. influenzae* (Group 4) the serum neopterin level ($13 \pm 8 \text{ nmol} \cdot l^{-1}$) was significantly elevated above the normal level found in Group 5 ($p < 0.05$). One to three weeks after the acute phase, the level had decreased to $8 \pm 6 \text{ nmol} \cdot l^{-1}$, and after 3–6 weeks it was $3 \pm 1 \text{ nmol} \cdot l^{-1}$, which was within the normal range.

Nine HIV-positive patients with bacterial pneumonia (Group 2) had a neopterin level of $41 \pm 34 \text{ nmol} \cdot l^{-1}$ at the acute stage, which was significantly elevated compared with the asymptomatic HIV-positive controls ($p < 0.05$), and the HIV-negative patients with bacterial pneumonia ($p < 0.05$). Four of the patients had AIDS, and the other five had HIV-related symptoms. Within 3–12 weeks after start of treatment, the level declined to $19 \pm 11 \text{ nmol} \cdot l^{-1}$; this change was not significant.

During the acute phase of *Pneumocystis carinii* pneumonia (PCP), the mean serum neopterin level was $39 \pm 27 \text{ nmol} \cdot l^{-1}$ in 30 subjects (Group 1). This level did not differ significantly from the level found in the HIV-positive patients with bacterial pneumonia. After start of treatment, the neopterin concentration declined significantly

Table 1. – Serum neopterin levels ($\text{nmol} \cdot l^{-1}$) in HIV-positive and HIV-negative subjects

Diagnosis	HIV-negative subjects	HIV-positive subjects
Asymptomatic	$4 \pm 3^{**}$ (1–12) n=48	9 ± 4 (1–20) n=38
Acute bacterial pneumonia	$13 \pm 8^{\dagger}$ (1–35) n=16	$41 \pm 34^{\dagger *}$ (6–110) n=9
Acute <i>P. carinii</i> pneumonia	39 ± 27 (9–125) n=30	

Data are presented as mean \pm SD, and range in parenthesis. HIV: human immunodeficiency virus. The bacterial pneumonias were caused by *S. pneumoniae* and *H. influenzae*. *, **: $p < 0.05$, < 0.001 respectively HIV-negative vs positive; \dagger : $p < 0.05$ asymptomatic vs infected.

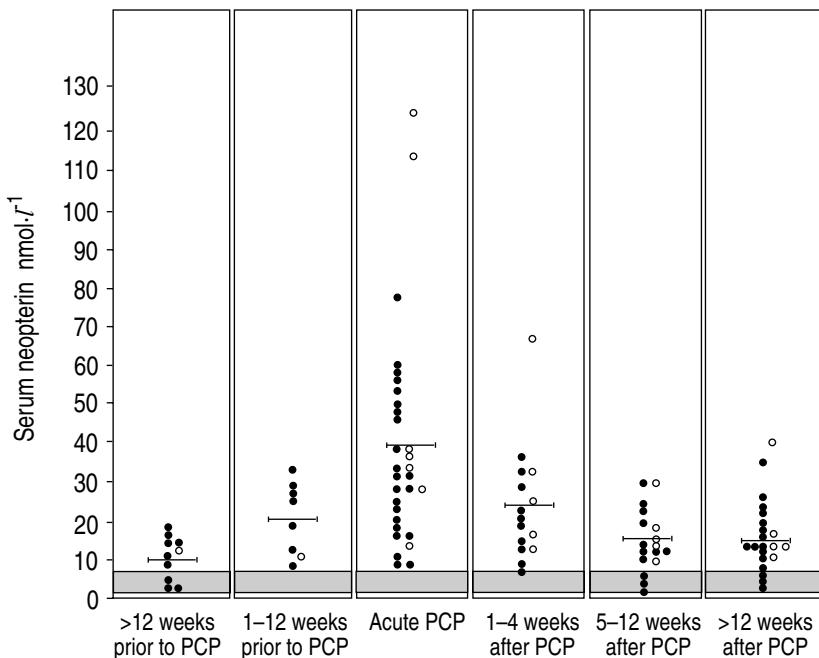


Fig. 1. – Changes in serum neopterin in HIV-positive (●) and AIDS (○) patients, who developed *Pneumocystis carinii* pneumonia (PCP) (Group 1). Shaded area represents normal range results. HIV: human immunodeficiency virus; AIDS: acquired immune deficiency syndrome.

($p<0.05$) within the first 4 weeks and fell further after 5–12 weeks (fig. 1). More than 12 weeks after PCP the level was $15\pm 9 \text{ nmol}\cdot\text{l}^{-1}$, which was significantly ($p<0.05$) higher than the level found prior to development of PCP in 10 patients ($10\pm 5 \text{ nmol}\cdot\text{l}^{-1}$). Exclusion of the two extreme outliers resulted in only small changes of serum neopterin in the acute stage ($34\pm 16 \text{ nmol}\cdot\text{l}^{-1}$) and within 4 weeks after the infection ($21\pm 8 \text{ nmol}\cdot\text{l}^{-1}$).

Ten patients who developed PCP were investigated prior to the lung infection. The neopterin level was $10\pm 5 \text{ nmol}\cdot\text{l}^{-1}$ >12 weeks before the PCP, which was not significantly different from the mean level in the HIV-positive controls. In 8 of the subjects, the neopterin concentration increased further to $21\pm 9 \text{ nmol}\cdot\text{l}^{-1}$ within the 12 weeks prior to the acute stage. In the 10 subjects the neopterin level increased significantly ($p<0.05$) to $38\pm 19 \text{ nmol}\cdot\text{l}^{-1}$ at the acute stage, and 12 weeks later the level had decreased significantly ($p<0.05$) to $14\pm 3 \text{ nmol}\cdot\text{l}^{-1}$.

The 23 patients with PCP as the first manifestation of AIDS had a mean serum neopterin value of $34\pm 18 \text{ nmol}\cdot\text{l}^{-1}$ at the acute stage, whereas the subgroup of seven AIDS patients with both PCP and another AIDS-defining disease had a level of $56\pm 41 \text{ nmol}\cdot\text{l}^{-1}$ at the acute stage of PCP.

Beta₂-microglobulin (table 2 and fig. 2)

HIV-negative controls had a serum β_2 -microglobulin concentration, significantly lower than that of asymptomatic HIV-positive subjects ($p<0.001$) (table 2). In 16 HIV-negative patients with acute community-acquired pneumonia due to pneumococci and *H. influenzae*, the mean β_2 -microglobulin level was $2.5\pm 0.7 \text{ mg}\cdot\text{ml}^{-1}$. One to three weeks later, it was $2.4\pm 0.8 \text{ mg}\cdot\text{ml}^{-1}$, and after 3–6 weeks it

Table 2. – Serum beta₂-microglobulin levels ($\text{mg}\cdot\text{ml}^{-1}$) in HIV-positive and HIV-negative subjects

Diagnosis	HIV-negative subjects	HIV-positive subjects
Asymptomatic	$1.5\pm 0.3^{**}$ (1.0–2.4) n=48	2.0 ± 0.6 (1.2–3.7) n=38
Acute bacterial pneumonia	2.5 ± 0.7 (1.2–3.6) n=16	$4.2\pm 1.6^{\dagger*}$ (2.5–6.9) n=9
Acute <i>P. carinii</i> pneumonia		3.7 ± 1.0 (2.1–7.7) n=30

Data are presented as mean \pm SD, and range in parenthesis. HIV: human immunodeficiency virus. The bacterial pneumonias were caused by *S. pneumoniae* and *H. influenzae*. *: $p<0.05$, $p<0.001$. HIV-negative vs positive; † : $p<0.05$ asymptomatic vs infected.

was $1.7\pm 0.3 \text{ mg}\cdot\text{ml}^{-1}$, which was in the upper part of the normal range.

Nine HIV-positive patients with acute pneumonia due to pneumococci and *H. influenzae* had significantly elevated levels of β_2 -microglobulin ($4.2\pm 1.6 \text{ mg}\cdot\text{ml}^{-1}$) compared with the HIV-positive controls ($p<0.05$), and the HIV-negative subjects with bacterial pneumonia ($p<0.05$). In the postinfectious period 3–12 weeks later, the serum concentration remained at $4.3\pm 1.2 \text{ mg}\cdot\text{ml}^{-1}$ in eight patients investigated.

In the 30 patients with acute PCP, the mean β_2 -microglobulin level was $3.7\pm 1.0 \text{ mg}\cdot\text{ml}^{-1}$, not significantly different from the level in HIV-positive patients with bacterial pneumonia. One to four weeks after start of treatment the level was $3.9\pm 1.2 \text{ mg}\cdot\text{ml}^{-1}$, and after 12 weeks the concentration ($3.6\pm 0.7 \text{ mg}\cdot\text{ml}^{-1}$) was significantly

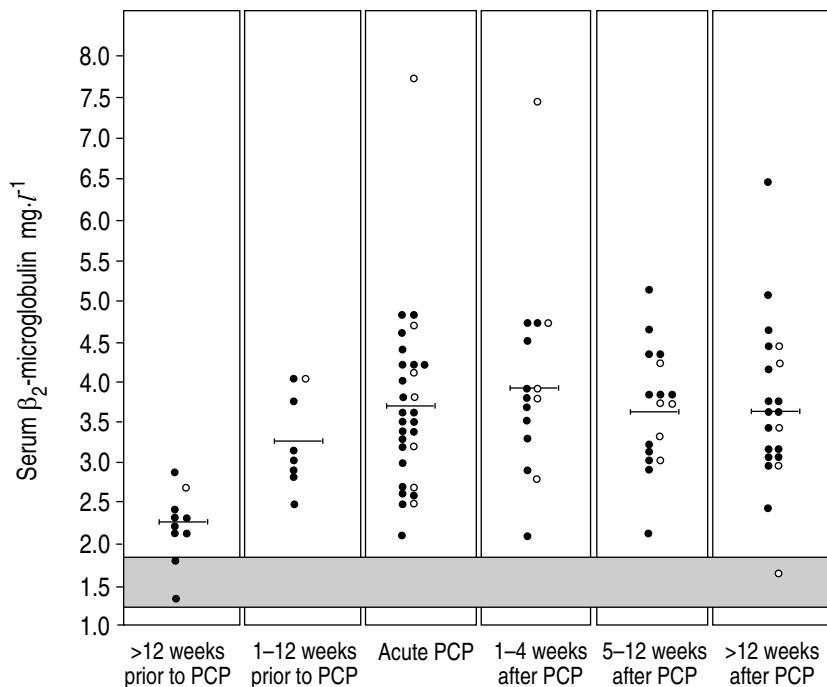


Fig. 2. – Changes in serum β_2 -microglobulin in HIV-positive (●) and AIDS (○) patients, who develop *Pneumocystis carinii* pneumonia (PCP). Shaded area represents normal range values. For abbreviations see legend to figure 1.

higher than the level found in 10 patients (2.2 ± 0.4 mg·ml⁻¹), 12 weeks prior to PCP development (fig. 2). By excluding the extreme outlier, the serum β_2 -microglobulin level changed only a little. The level was 3.6 ± 0.8 mg·ml⁻¹ at the acute stage, 3.7 ± 0.7 mg·ml⁻¹ within 4 weeks after the infection, and 3.5 ± 0.8 mg·ml⁻¹ after 12 weeks.

More than 12 weeks prior to the development of PCP, the mean β_2 -microglobulin level was 2.2 ± 0.4 mg·ml⁻¹ in 10 subjects, not significantly different from the level in the HIV-positive controls. Levels increased further to 3.3 ± 0.6 mg·ml⁻¹ in the 12 weeks prior to PCP. In the 10 subjects, the β_2 -microglobulin level was 3.4 ± 0.6 mg·ml⁻¹ at the acute stage, and was unchanged (3.4 ± 0.7 mg·ml⁻¹) 12 weeks after the acute infection.

In the seven HIV-positive patients with both PCP and other AIDS-defining disease the concentration of β_2 -microglobulin was 4.1 ± 1.6 mg·ml⁻¹ at the acute stage of PCP, whereas it was 3.6 ± 0.8 mg·ml⁻¹ in the remaining 23 patients, with PCP as the only AIDS-defining disease.

Discussion

In this study, we found that *Pneumocystis carinii* and encapsulated extracellular bacteria might stimulate the secretion of neopterin and β_2 -microglobulin. However, neopterin and β_2 -microglobulin did not seem to be of value in the distinction between PCP and bacterial pneumonia in HIV-positives, as the levels of the markers were within the same range. The results should be interpreted with caution, due to the small number of patients in one of the groups.

The small group of HIV-positive patients with bacterial pneumonia consisted of four AIDS patients and five

patients with HIV-related symptoms. The high concentrations of neopterin and β_2 -microglobulin at the acute stage and in the recovery phase may be caused by progressive HIV-infection and bacterial infection which stimulated the cellular immune system with increasing serum concentrations of the two markers. Increased levels of neopterin and β_2 -microglobulin were also measured in pneumonia in HIV-negatives, but the levels were significantly lower than the ones found in the HIV-positive patients. The mechanism behind the increased synthesis of neopterin and β_2 -microglobulin in bacterial pneumonia is unknown. Extracellular bacterial infections are not commonly linked with activation of the cellular immune system. However, NIEDERWIESER *et al.* [14] observed elevated neopterin in patients with staphylococcal pneumonia, and others [15, 16] found increased β_2 -microglobulin levels in cerebrospinal fluid in bacterial meningitis. The primary barrier against lung infection with extracellular bacteria depends on antibodies and phagocytosis by alveolar macrophages and polymorphonuclear leucocytes. Lipopolysaccharide from pneumococci and *H. influenzae* may induce release of interferons, which may provoke neopterin synthesis from the alveolar macrophages and increase the expression of β_2 -microglobulin on the surface of macrophages and leucocytes [17].

In patients, who later developed PCP, the levels of neopterin and β_2 -microglobulin were within the range found in HIV-positive controls more than 12 weeks prior to the acute pneumonia. The concentrations of both markers rose markedly within 12 weeks before subjects developed acute symptoms of lung infection. This could be explained by exacerbation of the HIV-infection, with increased viral replication or reactivation of silent viral pathogens, but it is also possible that a slow propagation of *P. carinii* in the alveolar lumen in the weeks before

the acute state may be responsible for the increasing level of the two markers by *in vivo* activation of the cellular immune system [10, 18]. Serum neopterin reached its highest level in the acute phase, and after start of treatment the concentration declined significantly, but it did not descend completely to premorbid level, despite disappearance of symptoms. The significant rise and subsequent decline of neopterin in close relation to PCP may indicate that the parasites stimulated the activated T-cells, and that the preactivated macrophages/monocytes *in vivo* had preserved responsiveness to interferon-gamma and the ability to secrete neopterin. Serum β_2 -microglobulin was significantly raised in the acute phase of PCP, and remained elevated despite treatment and recovery. In the postinfectious period, the reduction of the concentration was very slow, and the serum level did not correlate with the clinical activity of PCP. Cellular activation due to the progressive HIV-infection, and perhaps reactivated latent viral infections, may contribute to the persistently elevated levels of β_2 -microglobulin and neopterin after adequate treatment of PCP.

It may be concluded from our studies that neopterin and β_2 -microglobulin secretion may be stimulated by *Pneumocystis carinii* and extracellular bacteria in the HIV-positive patients. However, neither neopterin nor β_2 -microglobulin seem to be of value in distinction between PCP and bacterial pneumonia in HIV-positive patients. In HIV-negative patients with bacterial pneumonia the concentration of both serum markers may correlate with the clinical course of lung infection, whilst in patients with PCP only neopterin may partly correlate with the clinical activity. β_2 -microglobulin seems to be highly elevated above premorbid level more than 12 weeks after the PCP, despite recovery. The increased concentration may, in part, be explained by repeated infections and progression to AIDS.

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