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Expansion of neopterin and beta₂-microglobulin in cerebrospinal fluid reaches maximum levels early and late in the course of human immunodeficiency virus infection

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Summary. Elevated cerebrospinal fluid (CSF) levels of neopterin and beta₂-microglobulin (β 2MG) reflect activation of the cellular immune response in the central nervous system (CNS). In 118 consecutive subjects [15 controls and 103 patients with human immunodeficiency virus (HIV) infection classified according to the Walter Reed staging system (WR)], neopterin and β 2MG were determined in paired samples of CSF and serum. The permeability of the blood-CSF barrier and local release of neopterin and β 2MG were taken into account: The molecular weight and diameter were used to determine filtration at the blood-CSF barrier. CSF neopterin levels were increased in all stages of HIV infection. β 2MG levels were elevated in WR2 and later stages. Neopterin, β 2MG, and cell counts similarly showed peaks in WR2, as did neopterin and β 2MG also in the later stages WR5 and WR6. Neurologically asymptomatic patients exhibited higher neopterin CSF levels than did controls (12.67 ± 11.6 vs. 2.34 ± 1.05 nmol/l, $P < 0.001$) and higher CSF β 2MG (2.12 ± 1.25 vs. 1.3 ± 0.37 mg/l, $P = 0.001$). Patients with HIV encephalopathy had higher levels of β 2MG (3.75 ± 1.83 mg/l) than asymptomatic patients ($P < 0.01$). CSF levels of neopterin were markedly different in patients with HIV encephalopathy and toxoplasmosis ($P < 0.01$). A high quantity of local release of the markers neopterin and β 2MG may reflect HIV infection of the CNS in early and late stages and additional release upon opportunistic infections.

Key words: Human immunodeficiency virus infection – Neopterin – Beta₂-microglobulin – Cerebrospinal fluid – Cerebral toxoplasmosis

Abbreviations: AIDS = acquired immunodeficiency syndrome; β 2MG = beta₂-microglobulin; CNS = central nervous system; CSF = cerebrospinal fluid; HIV = human immunodeficiency virus (type I); RIA = radioimmunoassay; SD = standard deviation; SEM = standard error of the mean; WR = Walter Reed (staging classification); ELISA = enzyme-linked immunosorbent assay

Patients with early asymptomatic human immunodeficiency virus (HIV) infection often present with an inflammatory reaction of the cerebrospinal fluid (CSF), while apparent neurological symptoms are missing [2, 8, 19]. Cell count, albumin levels, neopterin and beta₂-microglobulin (β 2MG) levels have been reported to be elevated in asymptomatic HIV infection [15, 19].

Neopterin is produced by macrophages after stimulation with gamma-interferon during activation of the cell-mediated immune response [18, 19]. Serum concentrations of neopterin are elevated in HIV infection and have been demonstrated to convey prognostic information [1, 5]. Increased levels of neopterin have been found in patients with acute aseptic meningoencephalitis and during seroconversion to HIV [4].

Elevated levels of β 2MG reflect activation of the cellular immune response and an increased turnover of cell membranes. High CSF levels have been detected in patients with acute viral infections of the central nervous system (CNS) [15, 20] and in subjects with myeloproliferative disease with CNS involvement [14].

In an attempt to attain a more detailed description of the natural course of immune activation in the CNS, we started to determine neopterin and β 2MG levels according to stages of the Walter Reed (WR) staging classification [16]. Another aim was to compare the CSF levels in patients with HIV encephalopathy (term used synonymously with HIV dementia complex) in contrast with cerebral toxoplasmosis. Minor impairment of the blood-CSF barrier has been reported in HIV infection and HIV encephalopathy [2, 6]. The permeability of the blood-CSF barrier and local release of neopterin and β 2MG were estimated: The molecular weight and diameter were used to determine the expected filtration at the blood-CSF barrier and intracerebral production. We also calculated CSF/serum albumin quotients in order to estimate the range of barrier disorder.

Methods

Our study comprised 118 subjects, including 15 control subjects recruited from a group of HIV-1 antibody negative patients with noninflammatory CNS disease (age-matched). Eight patients were classified as WR stage 1 (WR1), 17 as WR2, 10 as WR3, 12 as WR4, 15 as WR5, and 41 as WR6. Neurological evaluation included a detailed history and neurological examination in all patients. Patients were enrolled consecutively. Neurological symptoms were not regarded as an inclusion criterion. Informed consent was obtained from all patients undergoing lumbar puncture. In neurologically symptomatic patients, radiologic imaging techniques (computed tomography and/or magnetic resonance depending on clinical findings) were performed as indicated by the clinical findings.

HIV encephalopathy was diagnosed when major disabling neuropsychological signs according to the Centers for Disease Control (CDC) definition were found. The diagnosis required thorough exclusion of opportunistic manifestations. Neopterin (radioimmunoassay, RIA, Henning, FRG), β 2MG (enzyme-linked immunosorbent assay, ELISA, Pharmacia, Sweden), and albumin were determined in paired samples of CSF and serum. Cell counts were taken from the first portion of the CSF after lumbar puncture. Statistical evaluation was performed by *t*-tests and analysis of variance. Differences were regarded as significant if $P < 0.05$ [3].

Results

To estimate the intrathecal production of neopterin and β 2MG, we calculated the filtration according to the barrier concept by K. Felgenhauer [7]. Neopterin has a molecular weight of 253.2 ($C_9H_{11}N_5O_4$) daltons. The estimated maximum hydrodynamic radius of this neutral compound is extrapolated to 6.5 Å (Dr. Nau, Göttingen, pers. comm., extrapolation of filtration rate of small molecules). The resulting serum/CSF distribution in a steady-state equilibrium therefore can be assumed to be a quotient of 40/1 [8]. Thus, 1/40 (2.5%) of the neopterin CSF concentration originates from serum, whereas the rest (97.5%) originates from the CNS ("corrected CSF neopterin").

β 2MG has a molecular weight of 16900 daltons. The serum/CSF equilibrium for small proteins ($\Rightarrow 20$ Å) is in the range of 100 or above [6]. Thus, less than 1% of the CSF β 2MG is expected to originate from the blood.

Neopterin in CSF (nmol/l) \pm SEM

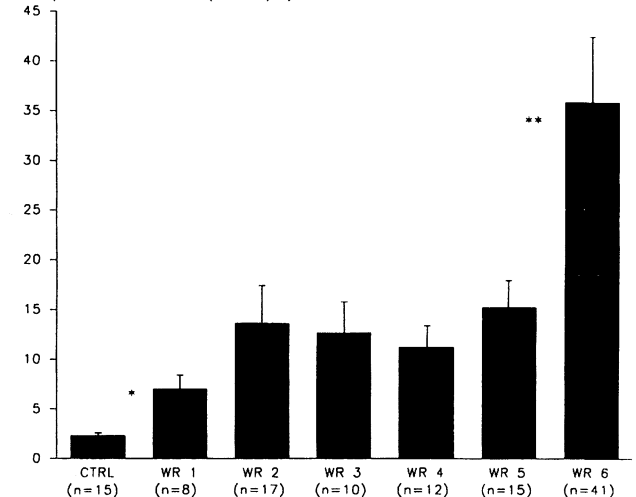


Fig. 1. Corrected neopterin level in cerebrospinal fluid (CSF) (=CSF neopterin minus 2.5% of CSF neopterin) according to the stages of the Walter Reed (WR) staging classification. CTRL=controls; SEM=standard error of the mean; * $P < 0.05$; ** $P < 0.01$

Neopterin Ratio CSF/serum \pm SEM

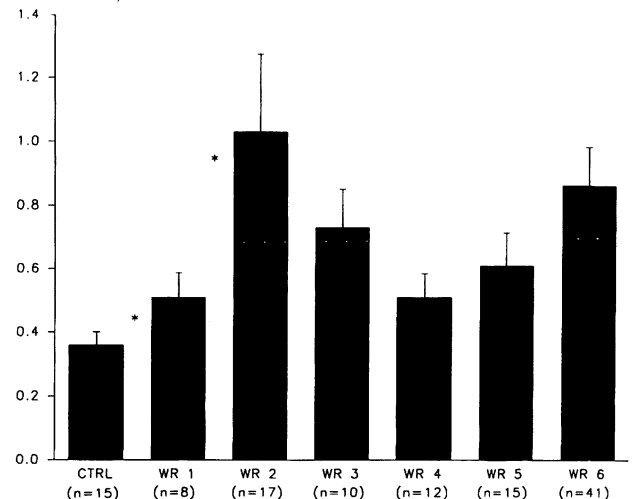


Fig. 2. CSF/serum ratio of neopterin according to the stages of the WR staging classification. * $P < 0.05$

CSF parameters according to WR stages

Corrected neopterin CSF levels were computed using the following formula: Neo Corr = neopterin in CSF minus 2.5% of neopterin in CSF. As compared with controls, the corrected CSF neopterin levels were elevated in all stages of HIV infection (WR1 versus controls with standard deviation: 7.02 ± 3.98 vs 2.34 ± 1.05 nmol/l; $P < 0.05$).

As shown in Fig. 1, the average level of CSF neopterin expands in WR2 but declines again in

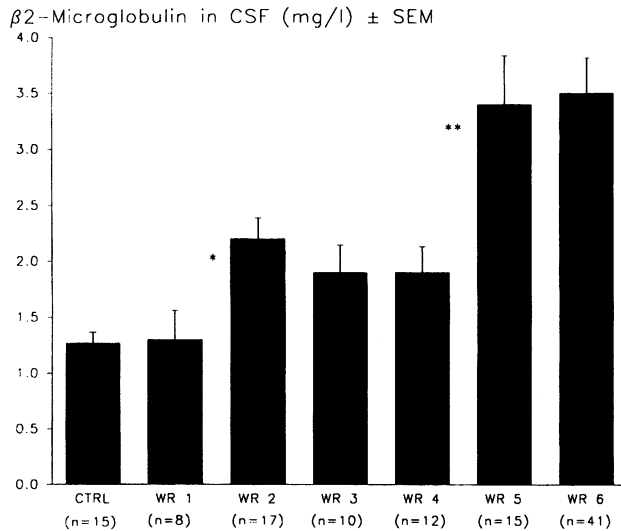


Fig. 3. Beta₂-microglobulin (β_2 MG) in CSF according to the stages of the WR staging classification. * $P < 0.05$; ** $P < 0.01$

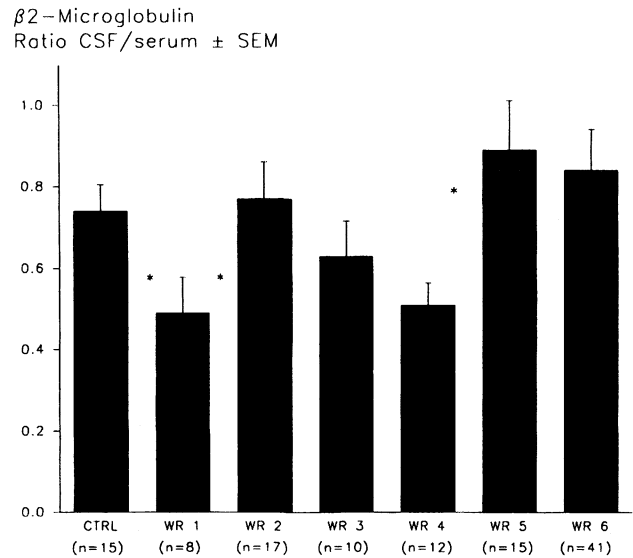


Fig. 4. β_2 MG in CSF and CSF/serum ratio according to the stages of the WR staging classification. * $P < 0.05$

Table 1. Cell count and total protein according to stages of the WR staging classification

	CTRL (n = 15)	WR 1 (n = 8)	WR 2 (n = 17)	WR 3 (n = 10)	WR 4 (n = 12)	WR 5 (n = 15)	WR 6 (n = 41)
Cell count (1/3)	5.3 \pm 3.8 *	13.5 \pm 14.7	37.8 \pm 39.8	41.8 \pm 43.8	18.5 \pm 18.2	15.7 \pm 13.6	15.5 \pm 30.8
Total protein (mg/dl)	33.7 \pm 18.9	34.5 \pm 12.4	40.7 \pm 15.5	40.9 \pm 14.1	33.7 \pm 7.0	41.9 \pm 20.1	47.3 \pm 22.6

* $P < 0.05$

WR3 and WR4. In the later stages WR5 and WR6, a significant increase of neopterin in the CSF and the ratio was observed (Fig. 1).

Quotients of CSF/serum neopterin increased significantly from controls (0.36 ± 0.16) to WR1 (0.51 ± 0.22) and from WR1 to WR2 (1.03 ± 1.01 , $P < 0.05$; Fig. 2).

According to molecule size (see above) and expected filtration estimates, 1% or less of the measured CSF β_2 MG is filtered from the serum to CSF. Albumin serum/CSF quotients ranged from 2.3×10^{-3} to 12.6×10^{-3} . Mean albumin quotients were 4.21×10^{-3} in WR1 ($\pm 2.73 \times 10^{-3}$) and 7.1×10^{-3} in WR6 ($\pm 6.86 \times 10^{-3}$; NS).

Thus, in WR6, the maximum increase of filtered β_2 MG in the CSF would not even double the serum portion of β_2 MG measured in the CSF (from 1% to 2%). Therefore, it seems acceptable to postulate that the actually measured CSF β_2 MG level equals basically the corrected β_2 MG taking into account the dysfunction of the blood-CSF barrier [which is not very pronounced in patients with full-blown acquired immunodeficiency syndrome

(AIDS) and opportunistic CNS infection; the upper limit in our data was 12.6×10^{-3}].

β_2 MG CSF levels (Fig. 3) showed no significant difference between WR1 and controls (1.30 ± 0.74 vs 1.27 ± 0.37 mg/l), while there was a rise in WR2 (2.2 ± 0.77 mg/l, $P < 0.05$). A further expansion occurred from WR4 to WR5 (1.9 ± 0.8 vs 3.4 ± 1.68 mg/l; $P < 0.01$). The ratio CSF/serum of β_2 MG was lower in WR1 than in controls (0.49 ± 0.25 vs 0.74 ± 0.24 ; $P < 0.05$). From WR1 to WR6 the ratio paralleled the two peaks for β_2 MG in WR2 as well as in WR5 and WR6 (Fig. 4).

Cell counts were elevated significantly compared with controls in all stages of HIV infection (Table 1). Albumin concentrations in the CSF reached a maximum in WR 6 (47.3 mg/l; Table 1).

CSF parameters according to neurological diagnosis

Neurologically asymptomatic patients showed higher levels for neopterin in the CSF (corrected) than did controls (12.67 ± 11.6 vs 2.34 ± 1.05 nmol/

Table 2. Corrected CSF neopterin (=CSF neopterin minus 2.5% of CSF neopterin) and CSF β 2MG and CSF/serum ratios, cell count, and total protein according to neurologic diagnosis in HIV-infected patients and controls (total patient number lower than 118 because of missing or uncertain diagnoses)

	Controls (n=15)		Asymptomatic HIV-infected (n=45)		HIV encephalo- pathy (n=15)		Cerebral toxo- plasmosis (n=9)		Toxoversus asymptomatic individuals
Corrected CSF neopterin (nmol/l)	2.34 \pm 1.05	***	12.67 11.6		21.40 \pm 20.4	*	73.90 61.3		*
Q neopterin (CSF/serum)	0.36 \pm 0.16	***	0.71 \pm 0.63		0.64 \pm 0.37		1.29 \pm 1.30		
CSF β 2MG (mg/l)	1.30 \pm 0.37	***	2.12 \pm 1.25	**	3.75 \pm 1.83	**	4.42 \pm 1.94		**
Q β 2MG (CSF/serum)	0.74 \pm 0.25		0.63 \pm 0.32		0.91 \pm 0.53		1.13 \pm 1.10		
Cell count (1/3 cells)	15.7 \pm 13.6		27.8 \pm 26.5		16.3 \pm 13.6		15.5 \pm 30.8		
Total protein (mg/dl)	33.7 \pm 18.9		37.3 18.6		41.7 \pm 32.5		63.2 \pm 85.2		

Q=quotient; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

l; $P < 0.001$). Similarly, β 2MG in the CSF was significantly expanded in HIV-infected subjects as compared with controls (2.12 ± 1.25 vs 1.3 ± 0.37 mg/l; $P < 0.001$; Table 2). Patients with HIV encephalopathy (diagnosis by clinical evaluation and exclusion of opportunistic manifestation) showed higher levels of neopterin in the CSF (21.4 ± 20.4 nmol/l; NS vs asymptomatic subjects) and β 2MG (3.75 ± 1.83 mg/l; $P < 0.01$ vs asymptomatic subjects).

In patients with cerebral toxoplasmosis the average (corrected) CSF level of neopterin was 75.8 ± 62.8 nmol/l, ranging significantly higher than in asymptomatic subjects and patients with HIV encephalopathy ($P < 0.05$).

β 2MG in the CSF was markedly elevated in cerebral toxoplasmosis ($P < 0.01$; Table 2).

Discussion

Increased CSF levels of neopterin and β 2MG have been reported in inflammatory neurologic disease, aseptic meningitis [20], Lyme borreliosis [4], measles [15], multiple sclerosis [20], neoplastic disease, and subarachnoid hemorrhage [13]. In individuals with HIV infection, intrathecal production of neopterin and β 2MG is detectable in the CSF [6, 7]. In comparison with normal subjects, HIV-infected patients show significantly increased levels of neopterin and β 2MG.

Earlier investigations by Fuchs et al. [9] and Sönnnerborg et al. [18, 19] suggested a rise in neop-

terin and β 2MG levels in the CSF according to clinical staging: asymptomatic HIV infection, lymphadenopathy syndrome, AIDS-related complex, and AIDS, respectively. Our data show that the increase of neopterin and β 2MG levels is not a continuum with respect to the stages of the WR staging classification. Rather, we find an early and a late expansion (Figs. 1–4).

The expansion of immunologic activation parameters in the early phases of HIV infection may be taken as an indirect sign of early CNS involvement, even though the patients may remain neurologically asymptomatic. Sönnnerberg and co-workers found the highest CSF levels of neopterin and β 2MG in patients in whom they successfully isolated HIV-1 from the CSF [19]. They found a ratio of CSF/serum of over 1.0 in patients with AIDS dementia complex. The mean ratio in our patients with this diagnosis was 0.73 for neopterin and 0.94 for β 2MG, again suggesting an intrathecal production by an activated immune system. In both parameters, a wide range of individual values contributed to a high variation.

One aim of our study was the comparison of CSF activation parameters in different clinical settings. Patients with overt neuropsychiatric symptoms like impairment of memory function, concentration, and coordination require a thorough diagnostic work-up. The diagnosis of HIV encephalopathy can only be made after the exclusion of opportunistic manifestations. A diagnostic tool administered by a spinal tap would be of importance. The hypothesis that CSF neopterin and

β 2MG might be significantly different in patients with either HIV encephalopathy or opportunistic infections could be confirmed by our data for the diagnosis of cerebral toxoplasmosis.

The high levels of intrathecally produced neopterin and β 2MG in HIV infection and neuro-AIDS may reflect an early infection and activation of cerebral compartments, e.g., cells of the microglia and imported macrophages. Current investigations in our group are concentrating on the measurement of neopterin in culture supernatants of microglia and astrocytoma cell lines infected with HIV.

CSF neopterin and β 2MG levels are parameters which reflect early CNS involvement in HIV infection. Mean maximum absolute levels and quotients (CSF/serum) are found in early and late stages of HIV infection.

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