Eur J Clin Chem Clin Biochem
1995; 33:559–562

Neopterin and β_2-Microglobulin as Serum Markers in a Placebo-Controlled anti-HIV Therapy Trial

By *Jan Carstens*[1], *Lars S. Teglbjaerg*[2] and *Finn T. Black*[1]

[1] *Department of Infectious Diseases, Marselisborg Hospital, University Hospital in Aarhus, Aarhus, Denmark*
[2] *Department of Infectious Diseases, Hvidovre Hospital, University Hospital in Copenhagen, Hvidovre, Denmark*

(Received January 23/June 6, 1995)

Summary: The purpose of this study was to evaluate the use of the biologic immune activation markers neopterin and β_2-microglobulin in monitoring human immunodeficiency virus (HIV)-positive patients without acquired immunodeficiency syndrome (AIDS) treated with isoprinosine and placebo. Serum samples obtained at the commencement of study and samples obtained after 24 weeks were available from 277 HIV-positive patients in the Scandinavian multicentre isoprinosine trial. After 24 weeks' treatment, the concentrations of β_2-microglobulin and neopterin had increased both in the isoprinosine group and the placebo group. However, in the isoprinosine group the relative increase within β_2-microglobulin was significantly smaller. Within neopterin, the increase from baseline level was small and not significantly different from the change in the placebo group. The β_2-microglobulin data might reflect a suppressive effect of isoprinosine on the HIV-induced activation of the cellular immune system. Because of the minor changes, there is no real evidence of neopterin and β_2-microglobulin being valuable as surrogate markers in monitoring therapy effects of isoprinosine.

Introduction

The variables available in evaluating anti-HIV treatments are limited. Since wishing a reaching of conclusions without waiting for survival differences to be established, a search for surrogate markers that might reflect therapeutic benefit early has been set up. Certain biological markers have been applied and of these the main part of attention has been given to the CD4+ cell count (1–4). Other biological markers include β_2-microglobulin and neopterin, which are markers of the activated cellular immunity (5, 6). Generalized immune activation occurs early in human immunodeficiency virus (HIV) infection and induces an increased production of neopterin and β_2-microglobulin, which persist throughout the infection (7, 8). These elevated levels of neopterin and β_2-microglobulin might be useful in monitoring the early in vivo efficacy of anti-HIV drugs on asymptomatic/mildly symptomatic HIV seropositive individuals. Only one single double-blind placebo-controlled study consisting of a limited number of subjects evaluated the effect of an anti-HIV drug on serum neopterin and β_2-microglobulin in mildly symptomatic HIV seropositive patients. Zidovudine reduced the immune activation, and neopterin and β_2-microglobulin appeared to be early and sensitive indicators of anti-HIV effects (9).

In retrospect we measured the levels of neopterin and β_2-microglobulin in 277 HIV-seropositive subjects included in the Scandinavian placebo-controlled trial of isoprinosine (10) in order to study the immune activation markers as early indicators of the anti-HIV effect of isoprinosine.

The Scandinavian isoprinosine study proved clinically beneficial on the isoprinosine-treated patients with a significant decrease in clinical progression towards acquired immunodeficiency syndrome (AIDS), while no evidence from the effect on either the CD4+ cell count or HIV-antigen level showed up (10, 11). As neopterin and β_2-microglobulin are markers with a rapid turnover, the hypothesis was, that changes in HIV activity induced by isoprinosine treatment might be detected more

rapidly with these sensitive markers, than by the well known CD4 count or clinical state.

Methods

Data and sera were collected from medical centres in Denmark and Sweden participating in the Scandinavian isoprinosine study (10) which was a randomized, double-blind, placebo-controlled multicentre trial. Originally the trial enrolled 866 HIV-positive patients without AIDS and aged 18–75 years. The treatment consisted of 1 g isoprinosine administered orally three times a day or matching placebo after an initial clinical evaluation. In no case side effects occurred during the treatment regimen continued for 24 weeks.

In this retrospective study, sera were collected from 19 out of 21 medical centres. Baseline serum samples and samples from week 24 were available from only 277 (139 isoprinosine, 138 placebo) of the originally enrolled patients. The paired sera of these 277 patients were all investigated in this study. The 139 patients in the isoprinosine group and the 138 patients in the placebo group were comparable in the non-significant differences in terms of sex, age, body weight, medical history within three months of study entry, physical examination, clinical staging, general well-being according to a visual-analogue scale, substrata according to CD4 cell count, and median number of baseline CD4 and CD8 lymphocytes. At baseline the isoprinosine group had a median CD4 count of 540 $\times 10^6$/l (range, 8–2100 $\times 10^6$/l) and the placebo group a median CD4 count of 510 $\times 10^6$/l (range 0–2600 $\times 10^6$/l). Nine percent of the study population (5% isoprinosine, 4% placebo) had a baseline CD4 count below 200 $\times 10^6$/l, while 40% (18% isoprinosine, 22% placebo) had a baseline CD4 count between 200–500 $\times 10^6$/l, and 51% (26% isoprinosine, 25% placebo) had over 500 $\times 10^6$/l. The median CD4 count in our selected group (n = 277) was not significantly different from the median CD4 count in the group of originally enrolled patients (n = 544) not taking part in this study.

At study entry, 94% in the isoprinosine group and 90% in the placebo group were assigned to CDC Group II or III, and 6% in the isoprinosine group and 10% in the placebo group were assigned to CDC Group IV without AIDS.

Serum samples obtained at study entry (baseline) and at the end of the treatment (week 24) were assayed for β_2-microglobulin with a solid phase time-resolved fluoroimmunoassay (Pharmacia DELFIA beta-2-microkit) and for neopterin with a radioimmunoassay (IMMUtest Neopterin, Henning Berlin GmbH).

Statistics

The MEDSTAT computer programme was used in analyzing the data. Neopterin and β_2-microglobulin differences between the isoprinosine group and the placebo group were assessed by the *Mann-Whitney* rank sum test. Correlations between the markers were determined by *Spearman* rank correlation test. Differences were considered significant if $p < 0.05$.

Results

At study entry, the isoprinosine and placebo groups had baseline median neopterin concentrations of 9.5 nmol/l (range, 0.5–43.0 nmol/l) and 10.5 nmol/l (range, 2.1–34.0 nmol/l), respectively. At baseline, the median β_2-microglobulin concentrations were 2.32 mg/l (range, 0.34–10.49 mg/l) in the isoprinosine group and 2.23 mg/l (range, 0.85–16.47 mg/l) (tab. 1) in the placebo group. The differences in baseline levels between the groups were not significant.

After 24 weeks of treatment the isoprinosine and placebo groups had higher median of neopterin and β_2-microglobulin. The isoprinosine group had a median neopterin concentration of 10.0 nmol/l (range, 3.1–40.7 nmol/l) and the placebo group had a concentration of 11.3 nmol/l (range, 3.6–57.2 nmol/l) (fig. 1). The relative increase within neopterin from baseline was not significantly different between the isoprinosine group and the placebo group. The median β_2-microglobulin level increased to 2.40 mg/l (range, 0.98–9.90 mg/l) in the isoprinosine group and to 2.57 mg/l (range, 1.13–18.47 mg/l) in the placebo group. The relative increase within β_2-microglobulin from baseline was significantly smaller in the isoprinosine group ($p < 0.05$). A significant positive correlation ($p < 0.001$) was observed between median neopterin and β_2-microglobulin levels at baseline and week 24 in both HIV groups.

After treatment, the median CD4 count accounted for 510 $\times 10^6$/l (range, 5–1800 $\times 10^6$/l) in the isoprinosine group and 505 $\times 10^6$/l (range, 0–2300 $\times 10^6$/l) in the placebo group. The absolute and relative differences in CD4 counts between the two groups were not significant. Between the isoprinosine and placebo groups, the difference in the number of patients whose condition progressed from CDC Group II or III to Group IV was not significant, and the difference in the frequency of night sweats, weightloss, temperature, chronic diarrhoea, oral candida, hairy leukoplakia, herpes zoster and herpes simplex infection was not evident.

Discussion

The purpose was to evaluate the use of neopterin and β_2-microglobulin as early surrogate markers in monitoring anti-HIV therapy in HIV-positive patients without AIDS. Since neopterin and β_2-microglobulin are mark-

Tab. 1 The median level of serum neopterin (nmol/l) and serum β_2-microglobulin (mg/l) before and after treatment with isoprinosine and placebo.

Marker		Isoprinosine	Placebo
Neopterin (nmol/l)	before	9.5	10.5
	after	10.0	11.3
β_2-Microglobulin (mg/l)	before	2.32	2.23
	after	2.40	2.57

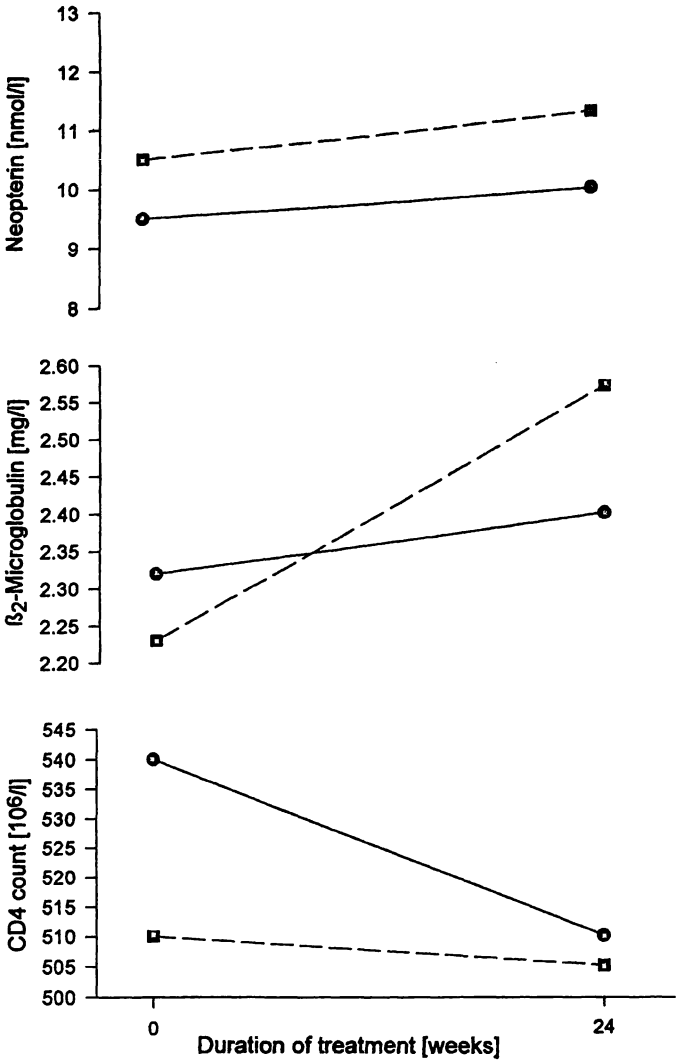

Fig. 1 Median levels of neopterin, β_2-microglobulin and CD4 counts at the commencement of the study and after 24 weeks' treatment. Solid-line: isoprinosine group, dashed-line: placebo group.

ers with a rapid turnover, we expected the changes in HIV activity induced by isoprinosine to be reflected rapidly with decreases in the levels of neopterin and β_2-microglobulin.

After 24 weeks' treatment, the levels of neopterin and β_2-microglobulin had increased from baseline in the isoprinosine and placebo groups. However, in the isoprinosine group the relative increase within β_2-microglobulin was significantly smaller. Within neopterin, the increase from baseline level was small but not significantly different from the change in the placebo group. Given the known strong sensitivity of the two markers in HIV-infected patients, isoprinosine surprisingly enough did not clearly affect neopterin and β_2-microglobulin. This might reflect a weak effect of isoprinosine on the activated HIV-infected cellular immune system. Because of the minor changes in the levels of the two markers, there is no real evidence of β_2-microglobulin and neopterin being valuable as surrogate markers in monitoring the therapy effect of isoprinosine.

A limitation is, that neopterin and β_2-microglobulin have been measured only at the commencement of the study and after 24 weeks' treatment. How isoprinosine affected the two markers in the early weeks of treatment is unknown. Perhaps, the two markers changed maximally early in the trial and later returned toward the levels in the placebo group as observed during treatment with azidothymidine (9).

The mechanism of action of isoprinosine is not clarified. It has been studied for several years, and its activity in vitro and in vivo seems to be due largely to a stimulating effect on the immune system while the antiretroviral activity is minimal (12–17). The increased levels of neopterin and β_2-microglobulin might reflect increased activation of the cellular immune system probably due to progression of HIV infection, despite isoprinosine therapy. However, the β_2-microglobulin results indicate that isoprinosine might have had a suppressive effect on the activation of the cellular immune system induced by HIV-infection. The increased levels of neopterin and β_2-microglobulin might also reflect conditions other than HIV-infection, such as opportunistic infections. However, the differences in the markers between the two HIV groups do not seem to be explained by differences in infections or other conditions, which may stimulate neopterin and β_2-microglobulin, since the two groups were comparable with non-significant differences in clinical staging, medical history and physical examinations.

In future, neopterin and β_2-microglobulin deserve to be closely evaluated as surrogate markers in a placebo-controlled trial with one of the new potent anti-HIV drugs and with a large number of asymptomatic or mildly symptomatic HIV-positive patients. An advantage of measuring neopterin and β_2-microglobulin rather than CD4 cell count or HIV antigen level is, that the two markers can be accurately quantified in serum of all subjects by simple and inexpensive assay techniques. In future trials, it would be of interest to determine whether those with the greatest activation marker response to anti-HIV treatment have greater clinical benefit. A correlation between an early effect of treatment on the immune activation markers and long term clinical outcome has not yet been reported in a controlled trial.

References

1. Jacobsen MA, Bacchetti P, Kolokathis A, Chaisson RE, Szabo S, Polsky B, et al. Surrogate markers for survival in patients with AIDS and AIDS related complex treated with zidovudine. Bio Med J 1991; 302:73–8.
2. Fischl MA, Richmann DD, Hansen N, Collier AC, Carey JT, Para MF, et al. The safety and efficacy of zidovudine (AZT) in the treatment of subjects with mildy symptomatic human immunodeficiency virus type 1 (HIV) infection. Ann Intern Med 1990; 112:727–37.
3. Stein DS, Korvick JA, Vermund SH. CD4+ lymphocyte cell enumeration for prediction of clinical course of human immunodeficiency virus disease: a review. J Infect Dis 1992; 165:352–63.
4. Choi S, Lagakos SW, Schooley RT, Volberding PA. CD4+ lymphocytes are an incomplete surrogate marker for clinical progression in persons with asymptomatic HIV infection taking zidovudine. Ann Intern Med 1993; 118:674–80.
5. Fuchs D, Hausen A, Reibnegger G, Werner ER, Dierich MP, Wachter H. Neopterin as a marker for activated cell-mediated immunity. Immunol Today 1988; 9:150–5.
6. Karlsson FA, Wibell L, Evrin PE. Beta-2-microglobulin in clinical medicine. Scand J Clin Lab Invest 1980; 40 Suppl 154:27–37.
7. Fahey JL, Taylor JMG, Detels R, Hofmann B, Melmed R, Nishanian P, et al. The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1. N Engl J Med 1990; 322:166–72.
8. Hofmann B. Neopterin and human immunodeficiency virus infection. Eur J Clin Chem Clin Biochem 1993; 31:191–5.
9. Bass HZ, Hardy WD, Mitsuyasu RT, Taylor JMG, Wang YX, Fischl MA, et al. The effect of zidovudine treatment on serum neopterin and beta-2-microglobulin levels in mildly symptomatic, HIV type 1 seropositive individuals. J Acquir Immune Defic Syndr 1992; 5:215–21.
10. Pedersen C, Sandström E, Petersen CS, Norkrans G, Gerstoft J, Karlsson A, et al. The efficacy of inosine pranobex in preventing the acquired immunodeficiency syndrome in patients with human immunodeficiency virus infection. N Engl J Med 1990; 322:1757–63.
11. Teglbjaerg LS, Kroon S, Sandström E, Moestrup T, Hansson BG, Vestergaard BF. Effect of isoprinosine on HIV antigenaemia. AIDS 1992; 6:199–201.
12. Tsang PH, Zanjani MD, Warner N, Bekesi JG. Restoration of impaired B- and T-lymphocyte subsets and functions in vitro by isoprinosine in prodromal homosexuals and AIDS patients. J Clin Lab Immunol 1986; 20:159–65.
13. Tsang PH, Sei Y, Bekesi JG. Isoprinosine-induced modulation of T-helper-cell subsets and antigen-presenting monocytes (Leu M3+Ia+) resulted in improvement of T- and B-lymphocyte functions, in vitro in ARC and AIDS patients. Clin Immunol Immunopathol 1987; 45:166–76.
14. Wiranowska-Stewart M, Hadden JW. Effects of isoprinosine and nPT 15392 on interleukin-2 (Il-2) production. Int J Immunopharmac 1986; 8:63–9.
15. Hansen JE, Mathiesen L, Pedersen C. No effect of isoprinosine on HIV infection in vitro. AIDS 1990; 4:1036–7.
16. Thorsen S, Pedersen C, Sandström E, Petersen CS, Norkrans G, Gerstoft J, et al. One-year follow-up on the safety and efficacy of isoprinosine for human immunodeficiency virus infection. J Int Med 1992; 231:607–15.
17. De Simone C, Albertini F, Almaviva M, Angarano G, Chiodo F, Costigliola P, et al. Clinical and immunological assessment in HIV+ subjects receiving inosine-pranobex. A randomised multicentric study. Med Oncol Tumor Pharmacother 1989; 6:63–7.

Dr. Jan Carstens
Langenaes Alle 41
DK-8000 Aarhus C
Denmark