

NEOPTERIN IN URINE AND SERUM AFTER KIDNEY TRANSPLANTATION

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Summary

Neopterin has recently been proposed as a valuable biochemical marker for the diagnosis of renal allograft rejection. The present study shows that serum neopterin levels increase under many circumstances: reduction of glomerular filtration rate, stimulation of endogenous production (i.e. rejection, infections), and depend on the nature of the treatment. Similarly, changes in urinary neopterin excretion are non-specific. Moreover, a loss of sensitivity was noted because of the high basal values which were observed even during the uncomplicated period. The monitoring of neopterin levels does not improve the diagnosis of rejection, but represents a useful parameter for retrospective studies and an indicator for other complications.

Introduction

Recent reports have suggested that the immune response is accompanied by increased urinary and serum levels of neopterin, a pteridine derived from GTP [1,2]. Consequently, the measurement of neopterin has been proposed as a biochemical marker of immunological complication after organ transplantation [3]. However, kidney transplantation represents a particular situation since renal function is known to influence pteridine excretion [4]. It is thus important to evaluate the limitations of this test and to define the possible factors interfering with an increase in serum and urinary neopterin in renal graft recipients.

Material and methods

Since pteridines are unstable, serum and urine samples were frozen immediately after collection. Neopterin in urine was measured after iodine oxidation by a high pressure liquid chromatographic (HPLC) method previously described [5]. Serum neopterin was determined by the radio-immunoassay developed by Rokos and co-workers [6]; reference values were those reported by the authors (5.3 ± 1.7 nmol/L).

Ninety-one healthy subjects formed the control group. Twenty-two renal allograft recipients have been studied. Urine and serum sampling were initiated on the day of transplantation and was continued until discharge. Two immunosuppressive regimens were used: azathioprine (3mg/kg/day) and prednisolone (1mg/kg/day, down to 0.25mg/kg/day within the first 3 months) in 14 patients; antilymphocyte antibodies (ALG) administration during the first two weeks was added to the previous protocol in eight patients. The treatment of rejection was with one gram doses of methylprednisolone (bolus) on three consecutive days. The first day of corticoid bolus was considered as the first day of a rejection crisis.

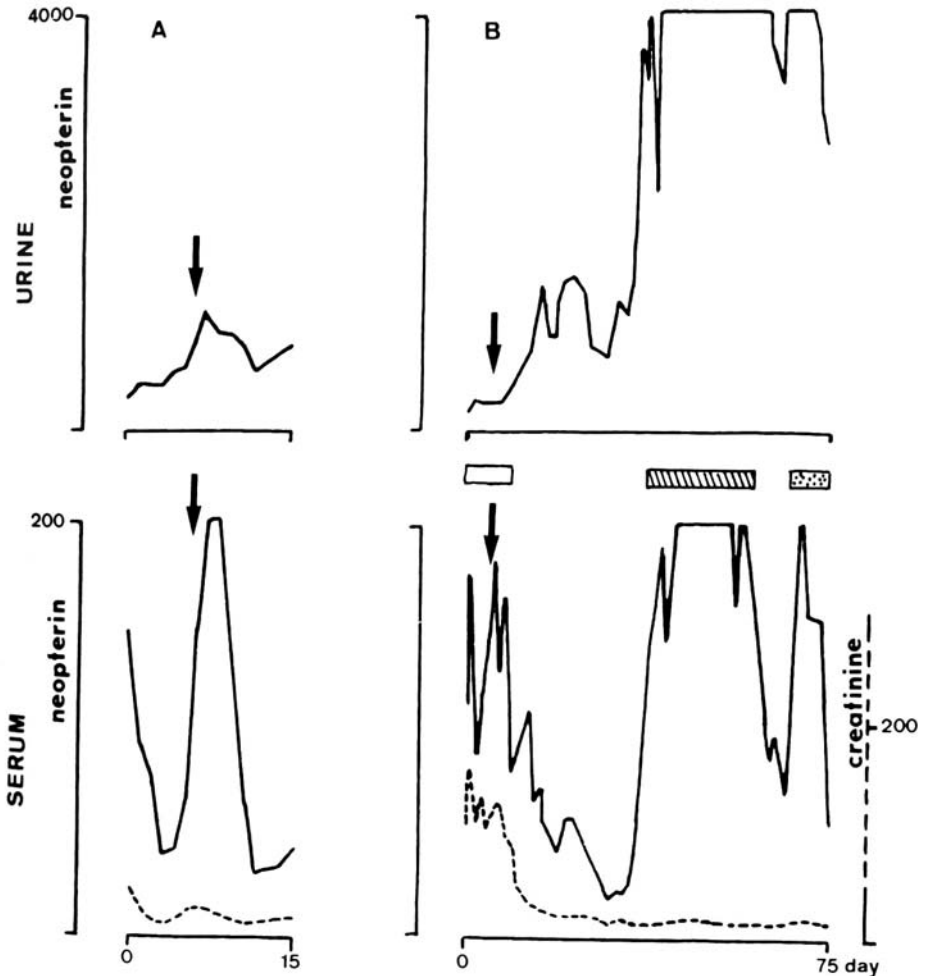


Figure 1. Neopterin in urine ($\mu\text{mol/mol}$ creatinine) and serum (nmol/L), and serum creatinine (mg/L) in two patients after renal transplantation \downarrow rejection episode, open square tubular necrosis, hatched square CMV infection, spotted square candida septicaemia

Results

The neopterin excretion in healthy controls was $312 \pm 94 \mu\text{mol/mol}$ of creatinine. The distribution of neopterin levels in 669 urine samples obtained from the 22 graft recipients showed that 91 per cent of values were above the normal range $\pm 2\text{SD}$.

During uncomplicated periods, the neopterin excretion was $1090 \pm 528 \mu\text{mol/mol}$ creatinine ($n=54$). In all of these cases, the serum neopterin concentration was found to be high (above 15nmol/L) and a significant correlation with serum creatinine was observed ($r=0.746$, $p<0.001$).

During rejection episodes a significant increase in urinary excretion and/or serum concentration of neopterin was observed in less than 50 per cent of cases. In most of these cases, a decrease in neopterin levels was observed within the few days following corticoid bolus treatment (Figure 1A).

In five patients, cytomegalovirus infection occurred and was accompanied by a significant increase of neopterin levels: 116 to $>200\text{nmol/L}$ in serum, 2100 to $45,000 \mu\text{mol/mol}$ creatinine in urine (Figure 1B). In one of these patients the cytomegalovirus infection was preceded by a rejection crisis which was itself responsible for an increase of neopterin, but despite the apparent clinical improvement after a corticoid bolus, neopterin continued to increase during the following days.

In patients receiving ALG, a progressive increase of neopterin excretion was observed before and after the cessation of ALG therapy (Figure 2A). In one case very high levels were noted before clinical signs of a serum reaction became evident (Figure 2B).

Discussion

The presence of neopterin in body fluids has been demonstrated only in humans and primates. Such a finding can be explained by a rate limiting biosynthesis of tetrahydrobiopterin, the most important unconjugated pteridine and the co-factor for aromatic aminoacid hydroxylation. Neopterin is a side product formed from non-enzymatic dephosphorylation and oxidation of dihydro-neopterin-triphosphate which is the true intermediate in the tetrahydrobiopterin biosynthetic pathway [7]. An increase of neopterin in serum and urine has been observed in various diseases involving immunological mechanisms (cancer, infectious diseases, autoimmune states, etc) [6,8]. The precise mechanisms for the neopterin increase remain unclear, but seem to be related to lymphocyte activation [2].

The present data confirm that neopterin increases are often associated with immunological complications. However, the diagnostic interest of such a 'marker' is limited by a lack of specificity and sensitivity. Any type of immunological response can be accompanied by increased levels of neopterin [2]. In the present study, the high neopterin levels observed during uncomplicated periods reflect in part the existence of a certain degree of allograft response despite the immunosuppressive therapy. The progressive increase of neopterin during antilymphocyte antibodies treatment can be similarly explained by the patient's immune

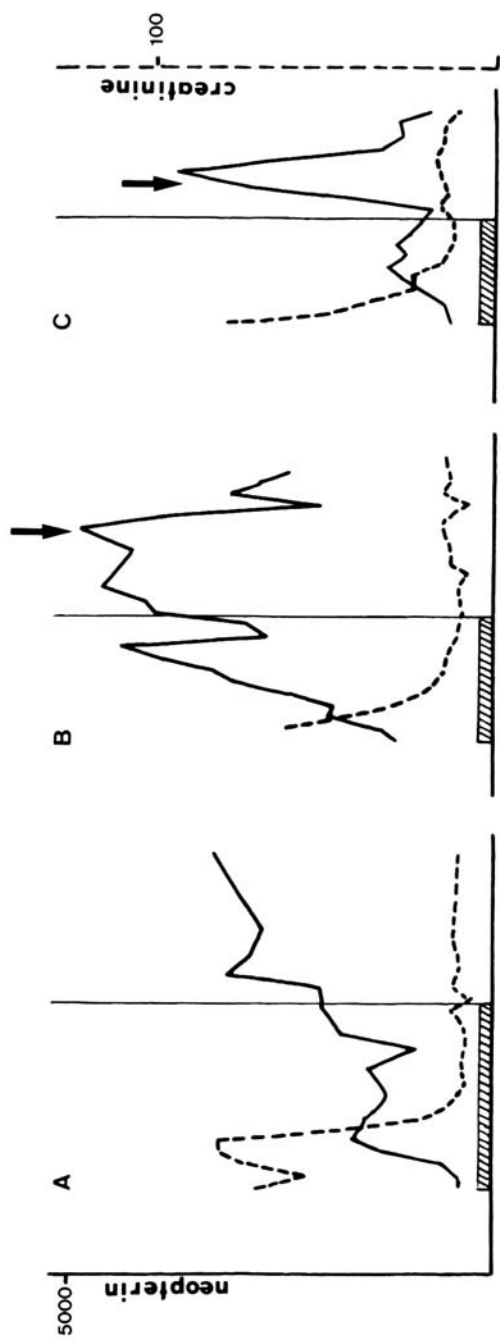


Figure 2. Urinary neopterin ($\mu\text{mol/mol}$ creatinine) and serum creatinine (mg/L) in patients who received antilymphocyte antibodies during the first two weeks. \uparrow rejection crisis, hatched square antilymphocyte antibodies administration

response against these antibodies. In such cases subsequent rejection crises are no more predictable by monitoring of neopterin levels. The finding of a correlation between serum neopterin and creatinine, together with the finding that neopterin clearance ($145 \pm 46 \text{ ml/min}$) in normal subjects is similar to that of creatinine, leads to the question of the reliability of neopterin changes if renal function is altered. For instance, during a rejection crisis it is sometimes difficult to link an increase of neopterin to the immunological reaction since such an event is usually accompanied by a significant reduction of glomerular filtration rate. In addition, high basal values of neopterin excretion were observed during the 'uncomplicated period' with no obvious cause for the increase such as alteration of renal function or stimulated endogenous production.

In our hands, the monitoring of serum or urinary neopterin concentrations provided a valuable aid for the retrospective analysis of immunological tolerance of the graft and events occurring during the follow-up. From a diagnostic point of view, neopterin did not appear as a 'marker' of immunological complications, as previously suggested [3], because of the absence of specificity contrasting with the need of specific therapeutic actions required to treat the different types of complication.

However, monitoring neopterin levels provided a good 'alarm sign'. For example, a persistent increase of neopterin excretion after a rejection crisis despite adequate treatment and normal renal function can direct attention towards the existence of a different complication (i.e. cytomegalovirus infection). On the other hand, a rapid and clear decrease might reflect the efficiency of the anti-rejection therapy.

However, further studies of an adequate number of graft recipients are required to establish the usefulness of neopterin measurement in the long term.

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