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Biliary neopterin for differentiation between liver allograft rejection and viral graft infection

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Differential diagnosis between rejection and infection of a liver graft still represents a major problem. Rising concentrations of neopterin in serum or urine sensitively indicate rejection or infectious complications after renal, cardiac or bone marrow transplantation [3]. Neopterin has been shown to be even more sensitive when measured locally, e.g. in the pancreatic juice of patients after pancreas allotransplantation [1]. In the present study, we measured urinary and, for the first time, biliary neopterin concentrations in nine liver allograft recipients during the early post-transplant period.

Key words: Liver transplantation – Rejection – Infection – Biliary neopterin

Materials and methods

Urine and T-tube bile samples were collected from two female and seven male patients after orthotopic liver transplantation over a period of 25 days on average. The underlying liver disease was chronic active hepatitis in five patients, alcohol-toxic cirrhosis in one and haemochromatosis in one. Prophylactic immunosuppression consisted of cyclosporin A, steroids and azathioprine. Rejection was diagnosed on the basis of clinical symptoms, impaired liver function, reduced bile production, and urinary neopterin excretion as well as graft histology. Acute graft rejection episodes were treated with boluses of methylprednisone as described elsewhere [2]. The urinary neopterin/creatinine ratio was measured in first-morning urine by high-performance liquid chromatography (HPLC). Biliary concentrations were determined according to a method previously described for assessment of neopterin in serum [4]. This technique uses solid-phase extraction combined with on-line elution from the solid phase onto the HPLC column. The method is also applicable for biliary specimens. Samples (50 µl) were diluted with aqueous sodium chloride (450 µl, 0.015 mol/l) and centrifuged at 10000 g for 5 min. The supernatant (100 µl) was mixed with 10 µl 0.1 mol/l $Fe^{3}P$ + EDTA solution and incubated for 20 min at room temperature. To 100 µl of this mixture, 10 µl phosphoric acid (5 mol/l) was added and 100 µl of the resulting mixture transferred to the solid-phase cartridge and processed further as described previously [4]. The biliary neopterin concentrations measured by HPLC were compared with results obtained with a commercially available radioimmunoassay (Henning-Berlin, Berlin FRG).

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Results

Increased urinary neopterin concentrations were seen to be associated with immunological and infectious complications such as acute rejection (n = 6), cytomegalovirus (CMV) infection (n = 2), hepatitis B (n = 1), hepatitis C (n = 1) and herpes infection (n = 1). During every rejection episode, rising biliary neopterin concentrations were found. The elevation in bile fluid and particularly the decrease in neopterin concentration subsequent to successful anti-rejection therapy were more pronounced and rapid than in urine. However, biliary neopterin concentrations began to increase about 24 h after urinary values started to rise. In contrast to their effect on urinary neopterin, CMV infection and hepatitis and herpetic infections were not associated with rising biliary neopterin concentrations.

In a patient with repeated rejection episodes resistant to various therapeutic attempts, biliary and urinary neopterin concentrations were invariably higher than in patients who responded well to anti-rejection treatment.

Discussion

The preliminary results of this study suggest that the determination of biliary neopterin concentrations facilitates differentiation of acute allograft rejection from viral infections after liver transplantation. Biliary neopterin appears not to increase in various forms of viral infections such as CMV, herpes and hepatitis. Since rejection can often not be distinguished from viral graft infection, even by histomorphology, measurement of biliary neopterin

may provide important information in addition to histology, clinical data and urinary neopterin.

The rise in urinary neopterin concentration preceded that in biliary neopterin concentration by 24 h. Elevated urinary neopterin seems to reflect the systemic activation of cellular immunity. It is remarkable, however, that viral infections, particularly viral hepatitis, did not cause a rise in biliary neopterin concentration.

Although not proven, it is very likely that hepatic neopterin is produced by Kupffer cells. It could be speculated that the delayed rise in neopterin levels in the bile fluid when compared with urinary neopterin is caused by a defective reticulo-endothelial system (RES). Its impaired function can be due to ischaemic damage and/or the fact that donor Kupffer cells are gradually replaced by recipient cells. Since hepatitis viruses are known to block the RES, no neopterin is produced during this type of infection.

If these results can be confirmed in a larger number of patients, biliary neopterin would be a very useful tool for diagnosing liver allograft rejection.

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