Biliary and Urinary Neopterin Concentrations in Monitoring Liver-Allograft Recipients

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Biliary neopterin concentrations were measured daily in nine liver-transplant recipients during the early post-transplant period (on average, 25 days). Concentrations increased strongly during rejection episodes and decreased quickly after successful antirejection therapy. Contrary to the changes of urinary neopterin, cytomegalovirus infection and hepatitis were not associated with an increase in biliary neopterin concentrations. Therefore, measurement of neopterin in bile fluid and in urine may aid in distinguishing rejection from infectious complications in liver-allograft recipients.

Additional Keyphrases: liver transplant • rejection • infection • cytomegalovirus • hepatitis • bile

Acute rejection of a liver allograft is difficult to distinguish from viral infection of the graft, such as hepatitis B or cytomegalovirus (CMV) infections. Increasing concentrations of neopterin in serum or urine sensitively indicate viral infections or rejection after renal, cardiac, and bone marrow transplantation (1) as well as after liver transplantation (2, 3). The use of urinary neopterin concentrations to predict long-term renal allograft survival was recently demonstrated (4). Increasing neopterin concentrations in body fluids indicate ongoing activation of the cellular immune system (1) and, thus, are not specific for viral infections or allograft rejections.

Neopterin is released by macrophages when stimulated with interferon-γ (5, 6), which is produced by activated T lymphocytes. Whereas viral infections activate cellular immunity systemically, activation occurs locally in rejection episodes. Therefore, neopterin may be more specific for rejection when measured locally. This was demonstrated, for example, by measuring neopterin in pancreatic juice after pancreas allotransplantation (7, 8). Moreover, the importance of local immune activation was also shown by measuring biliary soluble interleukin-2 receptors after liver transplantation (9).

In this pilot study, we sequentially measured urinary and, for the first time, biliary neopterin concentrations in nine liver-allograft recipients during the first post-transplant period to assess the effects of rejection and infection on neopterin concentrations in bile compared with those in urine.

Materials and Methods

Urine and T-tube bile samples were collected from nine patients after orthotopic liver transplantation for 25 days (on average), starting on day 6 (mean; range 3–8 days). The patients included two women and seven men (ages 33–55 years) presenting with end-stage liver disease caused by chronic active hepatitis (n = 6), alcoholic cirrhosis (n = 2), or hemochromatosis (n = 1). Prophylactic immunosuppression consisted of cyclosporin A, steroids, and azathioprine. Acute graft rejection was indicated by clinical symptoms; impaired liver function; increases in bilirubin, transaminases, and lactate dehydrogenase; reduced bile production as assessed with the T tube; and a strong increase in urinary neopterin excretion. Such rejection was confirmed by fine-needle aspiration (FNA). A positive biopsy by FNA was the trigger for treatment of acute graft-rejection episodes with 1–3-g boluses of methylprednisone. The kinetics of increasing neopterin (rather than an upper limit) are used as a sign for immunologic activation. Therefore, neopterin is measured daily. The increase of neopterin is an important and necessary sign complementing the other clinical and laboratory data. Neopterin increases as much as 4 days before the other indexes change.

CMV infection was diagnosed when antibodies to IgM appeared or titers of IgG antibodies to CMV increased at least fourfold within 2 weeks. Diagnosis of CMV disease was made from a combination of CMV serology with clinical symptoms, such as unexplained fever, increase in transaminases, cytopenia, intestinal pneumonitis, and ulceration of the gastrointestinal tract. CMV serology was performed by a quantitative enzyme-linked immunosorbent assay of IgG and IgM antibodies as described (10). Hepatitis B or C was diagnosed by clinical observations, determination of HBs and HBe antigens in serum, and histological evaluation of liver fine-needle biopsy.

The ratio of neopterin to creatinine in urine was determined in the first morning urine samples by reversed-phase HPLC (11, 12). Biliary concentrations of neopterin were determined by a method previously described for assessing neopterin in serum (13). In this technique, solid-phase extraction is combined with online elution from the solid phase onto the HPLC column. The method is also applicable for biliary specimens: samples (50 μL) are diluted with aqueous sodium chloride (450 μL, 0.015 mol/L) and centrifuged at 10 000 × g for 5 min. The supernate (100 μL) is mixed with 10 μL of 0.1 mol/L Fe³⁺-EDTA solution and incubated for 20 min at room temperature. Phosphoric acid (10 μL, 5 mol/L) is added to 100 μL of this mixture, and 100 μL is
then transferred to the solid-phase cartridge and processed further as described (13).

The biliary neopterin concentrations measured by HPLC were compared with results obtained with a commercially available radioimmunoassay (Henning-Berlin, Berlin, Germany).

Results

High urinary neopterin concentrations were associated with immunological and infectious complications, such as acute rejection (n = 6) and infection with CMV (n = 2), hepatitis B (n = 1), hepatitis C (n = 1), or herpes simplex (n = 1) (Figure 1, top). During rejection episodes, biliary neopterin concentrations also were higher (Figure 1, bottom). The increase in bile fluid was more pronounced, and the decrease of neopterin concentration after successful antirejection therapy was more rapid than in urine. However, biliary neopterin concentrations began to increase ~24 h after urinary values started to increase. The bile flow was not influenced by rejection except for one patient in whom a slight decrease was observed. In contrast to their effect on urinary neopterin, CMV, hepatitis B and C, and herpes simplex infections were not accompanied by increasing biliary neopterin concentrations.

Figure 2 summarizes biochemical and hematological findings of these patients. Liver-function tests, leukocyte numbers, and hemoglobin concentrations did not differ in the first days of rejection from the days before. Despite relatively high standard errors of the mean, we were able to determine that neopterin concentrations increased strongly in individuals. There were no episodes of acute rejection (n = 6) in the studied patients without increased biliary neopterin concentrations.

Two typical courses of neopterin concentrations in urine and in bile fluid are shown in Figure 3, one from a patient with a rejection episode and one from a patient with CMV infection. In both fluids neopterin concentration steadily increased over 3 days, which indicates immunological complication. CMV infections are not very common after transplantation. However, the decline of urinary neopterin concentrations (Figure 3, bottom) is accompanied by a strong increase in anti-CMV titers. This increase and decrease of neopterin, therefore, is caused by viral infection and not by other possible factors, such as those relating to the graft.

Discussion

This is the first study measuring neopterin in bile fluid. Our results suggest that the increase in the concentration of biliary neopterin is more pronounced than that of urinary neopterin during acute rejection episodes. Interestingly, biliary neopterin did not increase significantly during CMV, herpes simplex, and viral hepatitis B and C infections. In contrast, urinary and serum neopterin increased strongly during these
viral infections. Therefore, the concentration of biliary neopterin seems to be more specific than serum or urinary concentrations for differentiating acute allograft rejection from viral infections after liver transplantation. Because graft rejection often cannot easily be distinguished from viral infection, even by histomorphology, measuring biliary neopterin may provide new and important information in addition to the histological, clinical, and urinary neopterin data.

Viral infections that target the liver parenchyma result in dysfunction and diminished bile production. These defects are due to diminished uptake at the sinusoidal site and to lowered excretion at the canicular site. Therefore, a decrease in concentrations of biliary constituents, such as neopterin, and in bile production may occur. Thus, this lowered biliary secretion of neopterin would not parallel the changes of the neopterin concentrations in the circulation. However, the target of rejection is often the cells of the endothelium and the bile duct cells, because only these two cell types express class II antigens. Hepatocytes are less likely to be a target of rejection. Biliary concentration of most constituents and also of neopterin will decrease only in advanced states of rejection because of immunological injury to the vasculature and subsequent ischemia.

In rejection the increase in urinary neopterin concentrations preceded that of biliary neopterin concentrations by 24 h. An increased urinary neopterin is known to reflect the systemic activation of cellular immunity (1). Therefore, it is possible that lymphocyte activation begins outside the graft in the lymphatic system, and that activated mononuclear cells subsequently infiltrate the graft.

Our results are consistent and clear despite the small number of patients studied. Therefore, we suggest that concomitant assessment of urinary (or serum) and biliary neopterin could be used as a valuable tool for distinguishing rejection from infections after liver transplantation.

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References