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Case reports of interest

Usefulness of beta-D glucan in diagnosing *Pneumocystis carinii* pneumonia and monitoring its treatment in a living-donor liver-transplant recipient

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Abstract
Pneumocystis carinii pneumonia (PCP) is one of the fatal complications encountered after liver transplantation. The diagnosis of PCP is sometimes very difficult, because detection of the bacteria itself is not easy under some conditions, and the serum level of the chemical mediator is not yet considered to be a definitive diagnostic marker. We report a case of PCP that occurred 3 months after transplantation in a living-donor liver-transplant recipient; the disease developed during the course of outpatient follow-up when the patient's condition was stable. The patient was maintained with the usual level of immunosuppressants, using tacrolimus, steroid, and mycophenolate mofetil. The patient had a dry cough with mild fever, and a chest computed tomography (CT) scan showed a reticular shadow in the left lung field. The plasma level of beta-D glucan was high (135 pg/ml). We suspected an invasive fungal infection, but no pathogen was detected by routine fungal culture and cytology. Finally, *P. carinii* was detected by polymerase chain reaction (PCR), and we started treatment with trimethoprim-sulfamethoxazole (TMP/SMX) combined with an antifungal agent. During this period, the level of beta-D glucan correlated with the patient's clinical symptoms; this marker was very useful for monitoring the treatment of PCP in this living-donor liver-transplant recipient.

Key words Beta-D glucan · *Pneumocystis carinii* · Living-donor liver transplantation · PCR · Diagnosis

Introduction

Pneumocystis carinii pneumonia (PCP) is a potentially fatal complication in patients subjected to liver transplantation. In the absence of prophylaxis, the incidence of PCP for transplant recipients as a whole increased from 0.6% to 1.1% prior to 1990 to 9%–11.5% in more recent years.[1,2] The incidence of PCP in liver transplantation is 5%–10%[3] without prophylaxis, but this disease has been eliminated at most transplant centers by the prophylactic use of trimethoprim-sulfamethoxazole (TMP/SMX).[4] We report here a case of PCP that developed in the recipient 3 months after living-donor liver transplantation (LDLT). At this time the patient's plasma level of beta-D glucan was high. The patient was not administered with prophylactic agents because of drug-induced hepatotoxicity that occurred just after the transplantation. The diagnosis of PCP was difficult, but finally it was determined by polymerase chain reaction (PCR) of genetic material found in sputum. The high plasma level of beta-D glucan was considerably decreased by treatment with TMP/SMX. The plasma level of beta-D glucan may be useful as a diagnostic and therapeutic indicator. We discuss the usefulness of beta-D glucan in the diagnosis and therapeutic evaluation of invasive fungal infection in LDLT recipients.

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Case report

A 45-year-old man without any remarkable medical or familial history complained of general malaise, lack of appetite, and dark urine. A local physician diagnosed him as having acute viral hepatitis B with severe liver dysfunction, as well as the presence of ascites, and referred him to a neighboring hospital. However, his liver function worsened, demonstrating high levels of aspartate aminotransferase (AST; 4096 IU/l), alanine aminotransferase (ALT; 6063 IU/l), and total bilirubin (T-Bil; 11.8 mg/dl), as well as a prothrombin activity international ratio (PT-INR) of 4.20, and NH3, 387 μg/l. Plasma exchange (PE) was performed and he was referred to Tohoku University Hospital. Hepatitis B surface (HBs) antigen was negative, although IgM type anti-hepatitis B core (HBc) antibody was strongly positive, suggesting that the patient had acute hepatitis B virus (HBV) infection. His hepatic encephalopathy

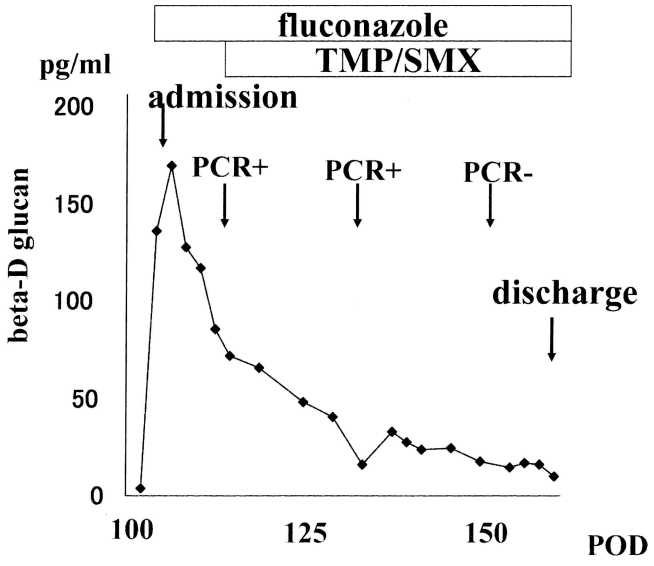

Fig. 1. Clinical course of *Pneumocystis carinii* pneumonia during diagnosis and treatment. *PCR*, polymerase chain reaction; *TMP*, trimethoprim; *SMX*, sulfamethoxazole; *POD*, postoperative day

Fig. 2. Chest computed tomography (CT) scan, showing diffuse ground-glass opacities and septal thickening, particularly in the upper lobe of the left lung

worsened from grade I to grade III after admission to our hospital, in spite of PE being performed three times. Abdominal computed tomography (CT) scan demonstrated apparent hepatic atrophy and moderate ascites. Using two Japanese models for the prognosis of fulminant hepatitis, we evaluated the prognosis, revealing an extremely poor prognosis and a mortality of 100%. Informed consent was obtained from his family, and LDLT was performed, with his wife as the donor, on the second day after admission. During his postoperative course, trimethoprim-sulfamethoxazole (TMP/SMX; TMP, 800 mg/day; SMX, 160 mg/day, twice a day) was withdrawn within 2 weeks, because graft biopsies twice revealed drug-induced liver damage. He was discharged on postoperative day (POD) 30 without any symptoms of infection.

While being followed-up as an outpatient, the patient presented with mild fever and dry cough and was again admitted to our hospital, on POD 113. He was maintained on tacrolimus (3.0 mg/day; trough level, 5–8 ng/ml), mycophenolate mofetil (MMF; 1500 mg/day), and methylprednisolone (8 mg/day) as immunosuppressive agents. On admission, his liver function parameters (LFTs) were within normal ranges, but the plasma level of beta-D glucan was very high (135 pg/ml); (Fig. 1). Sputum cultures yielded no bacteria or fungi. His chest radiograph showed no remarkable findings, but a chest CT scan revealed diffuse ground-glass opacities and septal thickening in the upper lobe of the left lung (Fig. 2). He was hypoxemic (PaO_2, 65.4 mmHg) but there were no abnormal findings on pulmonary perfusion scintigraphy. His serum was negative for *candida*, *aspergillus*, and *cryptococcus* antigens, as well as for cytomegalovirus and human herpes virus 6. However, PCR of sputum collected on the eighth day after admission was positive for *P. carinii*. Then we started to administer TMP/SMX (TMP, 1600 mg/day; SMX, 320 mg/day) combined with fluconazole (200 mg/day). He presented slight deterioration of renal function (serum creatinine, 2.3 mg/dl), but this improved with hydration. On the thirty-third day after admission, PCR of genetic material in a sputum sample was negative, but he still had a dry cough and was positive for beta-D glucan. On the fifty-sixth day, he was discharged without any symptoms, and his serum was negative for beta-D glucan.

Discussion

PCP has become a relatively rare infection in liver-transplant recipients, thanks to the standardization of primary prophylaxis with TMP/SMX.[5] *Pneumocystis carinii* has been considered as a protozoan, but it is more closely related to fungi than to protozoa on the basis of gene homology.[6] It typically manifests as fever accompanied by nonproductive cough, hypoxemia, and diffuse interstitial infiltration.

As P. carinii is more closely related to fungi than to protozoa, the measurement of plasma beta-D glucan, which is a cell-wall constituent of fungi, may be useful for both the diagnosis and the therapeutic monitoring of PCP.[7] Although plasma beta-D glucan is considered very useful in the diagnosis of PCP, there have been no reports on its usefulness in recipients of LDLT. We have reported here a case of PCP occurring 3 months

after LDLT, in which the plasma level of beta-D glucan was useful as a diagnostic and therapeutic indicator. However, although PCR of genetic material in bronchoalveolar lavage (BAL) fluid is particularly sensitive for the diagnosis of PCP,[8] BAL is not easy to perform in LDLT recipients with PCP and subrespiratory failure. Thus, other markers are needed to support the diagnosis of PCP. Previously, it was reported that a high plasma level of beta-D glucan (median value, 494 pg/ml) was detected in six patients with PCP.[9,10] In our patient, not conventional culture, but PCR of genetic material in sputum, and the peck value of beta-D glucan (135 pg/ml) were of diagnostic value. Moreover, beta-D glucan was a useful therapeutic indicator in our patient. In Japan, beta-D glucan is well recognized as a marker of invasive fungal infection. So we also administered fluconazole for the treatment of a hidden or combined fungal infection.

PCP prophylaxis with TMP/SMX is the standard regimen for LDLT recipients.[4] TMP/SMX prevents PCP in almost 100% of immunosuppressed patients, but its use is limited by the high incidence of adverse effects, such as bone-marrow suppression, skin manifestations, and liver/kidney dysfunction. From 9% to 40% of transplant recipients do not tolerate TMP/SMX, although intolerance is often not well documented.[11,12] Among alternative agents for treating the population of transplant recipients, inhaled pentamidine[13] and atovaquone[14] have been associated with frequent instances of therapeutic failure. In our experience, our patient was intolerant to TMP/SMX because of hepatotoxicity, proven by graft biopsy. We were not certain that TMP/SMX was the definitive reason for the elevation of liver function parameters, but we suspected it as one of the causes. We think that alternative agents such as atovaquone should be administered, but other agents might also induce liver dysfunction. Thus, we did not prescribe any medicines for preventing PCP. In retrospect, we should have prescribed some prophylactic agents after the values for liver function parameters normalized.

TMP/SMX is the drug of first choice for the treatment of PCP because of the availability of both intravenous and oral formulations of the drug, and because the serum levels attained with the orally administered drug are equivalent to the levels attained with the intravenous formulation in patients with a normal gastrointestinal function. Moreover, this drug is of both therapeutic and prophylactic value. Furthermore, TMP/SMX may be effective against *Listeria*, most *Nocardia* species, and many of the common bacterial pathogens, including encapsulated and unencapsulated gram-negative and gram-positive organisms. Second-line agents include intravenous pentamidine and dapsone-trimethoprim. In our patient, TMP/SMX was effective for the treatment of PCP, but it took around 2 months for clinical symptoms to disappear and for beta-D glucan to become negative.

In conclusion, we experienced a case of PCP that was diagnosed by PCR of genetic material in sputum; also, the patient's plasma level of beta-D glucan was useful as a diagnostic and therapeutic indicator. More studies of early noninvasive equantitative markers for PCP, such as beta-D glucan, are needed. And we have many things to do for the improvement of LDLT.[15–17]

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