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Disease Course in Childhood Steroid Sensitive Nephrotic Syndrome is Changeable?

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The long-term prognosis of steroid-sensitive nephrotic syndrome is relatively good. However, most patients experience relapses in their clinical course. In this issue, Sinha, *et al.* [1] reported the prognosis of steroid-sensitive nephrotic syndrome in 1071 children, which is the largest retrospective cohort study to date. They described that approximately one-half of the patients developed frequent-relapsing nephrotic syndrome (FRNS) or steroid-dependent nephrotic syndrome (SDNS), consistent with the rates in previous reports. FRNS is defined as occurrence of e"2 relapses in 6 months or e"3 relapses in 12 months, while SDNS is defined as two consecutive relapses while on a tapering dose of prednisolone. FRNS and SDNS are critical issues for children, because of the severe adverse effects of steroid treatment, such as short stature, glaucoma, and osteonecrosis. Psychological issues also occur in such patients owing to long hospitalization, frequent hospital visits, and side effects of treatment. Therefore, clarification of risk factors for a frequent-relapsing course and its prevention are quite important. The authors concluded that early onset (<4 years), lack of adequate initial therapy (<8 weeks), and short duration of initial remission lasting <6 months are risk factors for a frequent-relapsing course. Among these, the only one in which we can intervene is initial therapy. Although many pediatric nephrologists think that initial steroid treatment at disease onset may influence the future clinical course, the optimal length of initial steroid therapy remains controversial. A standard basic initial steroid therapy at primary onset was proposed by the International Study of Kidney Disease in Children (ISKDC) more than 30 years ago. The proposal was daily steroid therapy (60 mg/m^2/d) for 4 weeks followed by alternate-day treatment (40 mg/m^2/2d) for 4 weeks (original was 3 days of medication followed by 4 days off in a week, but later modified). Thereafter, this protocol was revised in a variety of ways to obtain a better prognosis.

The KDIGO (Kidney Disease: Improving Global Outcomes) guideline recommends that oral prednisone should be administered as a single daily dose starting at 60 mg/m^2/d or 2 mg/kg/d (maximum: 60 mg/d) for 4-6 weeks followed by alternate-day medication at 40 mg/m2/2d or 1.5 mg/kg/2d (maximum: 40 mg/2d) with tapering of the dose for 2-5 months [2]. The published US guideline recommends that the initial steroid therapy should be 2 mg/kg/d for 6 weeks followed by 1.5 mg/kg/2d on alternate days for 6 weeks (maximum: 40 mg/2d) [3]. In the Cochrane systematic review, it is stated that duration of therapy up to 7 months appeared to be more effective than therapy for 2 months in achieving sustained remissions. The authors recommended a further well-designed and adequately powered randomized controlled trial (RCT) to compare daily therapy for 4 weeks followed by alternate-day therapy for 6 months with the standard 2-month therapy [4]. In Japan, an RCT comparing daily therapy for 4 weeks followed by alternate-day therapy for 6 months with the standard 2-month therapy is ongoing. The results of this RCT will provide some suggestions regarding the optimal mode of initial steroid treatment. The duration of daily steroid therapy, i.e. 4 or 6 weeks, should also be evaluated by an RCT.

Although half of the 1071 patients progressed to FRNS, 185 children had FRNS or SDNS and 42 had late steroid resistance in long-term observation. However, 72% of FRNS patients were in remission or had infrequent relapses with immunosuppressive agents or low-dose steroid. Therefore, the long-term prognosis is relatively good. However, a certain rate of patients showed disease transition to the adult form or a difficult clinical course resistant to existing therapy. Such patients were likely to be complicated with severe infection, resulting in high mortality. Recently, rituximab, an anti-CD20 antibody, has become an emerging therapy for difficult nephrotic syndrome [5,6]. Rituximab allows such patients to discontinue steroid treatment and dramatically reduces the number of relapses. Rituximab can also induce remission in patients with intractable steroid-resistant nephrotic syndrome. Although more clinical experience is needed to establish a safe and effective mode of administration, such new molecular target therapies will improve the clinical course of childhood nephrotic syndrome in the future.

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BRIEF REPORT

Thrombotic microangiopathy due to multiple autoantibodies related to antiphospholipid syndrome

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Abstract
Background Antiphospholipid syndrome (APS) is a rare disorder in children. More than half of childhood APS occurs as secondary APS complicated by systemic lupus erythematosus (SLE) and other autoimmune diseases.
Case-Diagnosis/Treatment We encountered a boy with SLE who presented with thrombotic microangiopathy (TMA) due to APS. He was initially diagnosed with SLE and treated with methylprednisolone pulse therapy. However, his renal function rapidly deteriorated. Since poikilocytes were detected, we suspected that his condition was complicated by TMA or APS. Urgent plasma exchange, continuous hemodialysis, and intravenous cyclophosphamide saved the patient and his renal failure ameliorated. A renal biopsy performed at the onset of disease showed multiple microvascular thrombi, diffuse mesangiolysis, and cortical necrosis compatible with TMA. He was positive for anticardiolipin antibody, anti-β2-glycoprotein I antibody, and lupus anticoagulant as well as anti-phosphatidylserine-prothrombin complex IgG antibody (aPS/PT). Anti-a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13 (ADAMTS13) antibody was negative and ADAMTS13 activity was normal. The aPS/PT is thrombogenic and is a newly discovered lupus anticoagulant.
Conclusions Childhood TMA due to APS has rarely been reported. To the best of our knowledge this is the first report of pediatric TMA due to APS with positive aPS/PT. Physicians need to be aware of aPS/PT in pediatric APS and/or SLE.

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Introduction

Thrombotic microangiopathy (TMA) is a microvascular occlusive disorder predominantly characterized by platelet thrombi in renal and neurological tissue and other organs. Hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) are the clinical entities comprising TMA, with predominantly renal manifestations in the former, while the latter often presents with neurological and systemic manifestations. TTP and HUS are similar and clinical overlap is common. Systemic lupus erythematosus (SLE) is infrequently complicated by TMA. TMA occurs in 2–3% of adult lupus cases [1]. Most cases of TMA with concomitant SLE are caused by von Willebrand factor (vWF) cleaving metalloprotease (a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13: ADAMTS13)-related TTP or antiphospholipid antibody syndrome (APS) [2–4]. Half of childhood APS occurs as secondary APS complicated by another underlying disease such as SLE [5]. Lupus anticoagulant (LA), anti-β2-glycoprotein I antibody (β2GPI), and anticardiolipin antibody (aCA) were found to be positive in 22%, 40%, and

44% of pediatric lupus patients respectively [6]. We report a boy with rapidly progressive TMA due to SLE and APS (SLE/APS). Interestingly, he was positive for not only aCA and LA, but also anti-phosphatidylserine-prothrombin complex antibody (aPS/PT) [7]. A new lupus anticoagulant, the thrombogenic autoantibody aPS/PT, was recently discovered and has similar diagnostic value in APS [8]. In addition, it is known to cause TMA in adults [9]. We report the first pediatric case of TMA associated with APS that was positive for aPS/PT.

Case report

A 9-year-old Japanese boy with SLE was transferred to our hospital because of acute renal and cardiac failure. The chief complaints were oliguria, dyspnea, hypertension, and heart failure. He had no remarkable health problems or a family history of rheumatic diseases. His SLE was diagnosed at the age of 9 years and 11 months based on fever, butterfly-shaped erythema, pancytopenia, low serum complement, serum anti-dsDNA antibody (273 IU/mL; normal levels, <20 IU/mL), and antinuclear antibody (ANA). He also had high titer of anticardiolipin IgG antibody (120 IU/mL; normal levels, <10 IU/mL), and LA (3.4; normal levels, <1.3). The patient was initially treated with two courses of methylprednisolone pulse therapy (MPT). However, the renal function began to deteriorate (creatinine [Cr], 1.16 mg/dL; blood urea nitrogen [BUN], 85 mg/dL) and he developed oliguria. Before MPT, APTT was prolonged to 85 s and aCA (21.6 U/mL) was positive. The patient was transferred to the intensive care unit in our hospital 1 month after the onset of SLE.

On admission, tachycardia (125/min), dyspnea (40/min), and hypertension (138/90 mmHg) were observed. Palpebral conjunctiva was anemic but not icteric. Scattered purpura was present on the lower edematous extremities. Heart and respiratory sounds were normal. The abdomen was soft but distended. The patient presented with anemia (Hb, 10.5 g/dL) and thrombocytopenia (platelets, $7.0\times10^4/\mu L$). Poikilocytes were detected in the peripheral blood. D-dimer was elevated (3.1 μg/mL; normal levels, <1.0 μg/mL). Renal function had deteriorated (BUN, 92 mg/dL; Cr, 2.2 mg/dL; cystatin C, 5.02 mg/L) (Fig. 1). Phosphorus and potassium levels were increased because of renal failure. Lactate dehydrogenase (LDH, 505 IU/L; normal levels, <250 IU/L) levels were also increased. The patient had hypocomplementemia (C3, 30 mg/dL [normal levels, >70 mg/dL]; C4, 3 mg/dL [normal levels, >12 mg/dL]; CH50, <10 IU/mL [normal levels, >35 IU/ml]). ANA×160, anti-dsDNA antibody (42 IU/mL; normal levels, <12 IU/mL) and anti-ssDNA antibody (306 IU/mL; normal levels, <25 IU/mL) were positive. With regard to antiphospholipid syndrome, β2GP1 (21.6 U/mL; normal levels, <3.5 U/mL), aCA (52 U/mL; normal levels, <1.3 U/mL), and LA (1.97; normal, <1.3) were positive. Urinalysis showed hematuria and proteinuria (urinary protein, 85 mg/dL; urinary Cr, 35 mg/dL). Abdominal ultrasound revealed bright and swollen kidneys, decreased renal blood flow, and a moderate amount of ascites. No renal thrombosis was detected in the renal artery and vein. A chest X-ray showed a cardiothoracic ratio that had increased by 60% and pleural effusion, suggesting heart failure. Cardiac ultrasound showed a markedly decreased left ventricular ejection fraction (43%), dilated ventricular end diastolic diameter, and trivial mitral and tricuspid valve regurgitation. However, troponin T and creatinine kinase levels were normal.

There were no abnormal findings on brain magnetic resonance imaging (MRI) and or on an electrocardiogram. However, brain single photon emission computed tomography (SPECT) revealed a mild to moderate diffuse decrease in cerebral blood flow. The results of laboratory and radiological tests suggested TMA. We suspected that the patient suffered from SLE with concomitant TTP or renal thrombosis due to APS. We were not able to determine anti-ADAMTS13 antibody results on admission. However, while the cause of his condition was unknown, his renal function had rapidly deteriorated, and he needed urgent and aggressive therapy against TMA. Plasma exchange and continuous hemodialysis were promptly initiated. MPT followed by 500 mg/m^2 of intravenous pulse cyclophosphamide (IVCY) were also administered (Fig. 1). The first renal biopsy performed 6 days after admission showed multiple microvascular thrombi, diffuse mesangiolysis, ruptured glomeruli and cortical necrosis compatible with TMA (Fig. 2). Evaluation of International Society of Nephrology/ Renal Pathology Society (ISN/RPS) grading for lupus nephritis was not possible because of diffuse mesangiolysis and severe damage to glomeruli due to TMA. IgG, IgA, IgM, C3, C4, and C1q deposits were positive according to immunofluorescence staining. After the second course of MPT, the platelet count began to increase and urination had improved. Mycophenolate mofetil (MMF) was also started. Hemodialysis was continued for 2 weeks and plasma exchange was performed three times. One month after admission, we obtained the results of TMA-related laboratory tests. Surprisingly, ADAMTS13 activity and vWF activity on admission were normal. Anti-ADAMTS13 antibody was negative. In addition, factor H activity was normal and anti-factor H antibody was negative. Interestingly, anti-aPS/PT IgG antibodies were strongly positive (>50 U/mL; normal levels, >2 U/mL). We concluded that the patient's TMA was related to APS.

The second kidney biopsy performed 6 months after the onset of disease revealed the disappearance of active lesions such as microvascular thrombi and mesangiolysis. Chronic lesions such as tubular atrophy, interstitial fibrosis, and globally

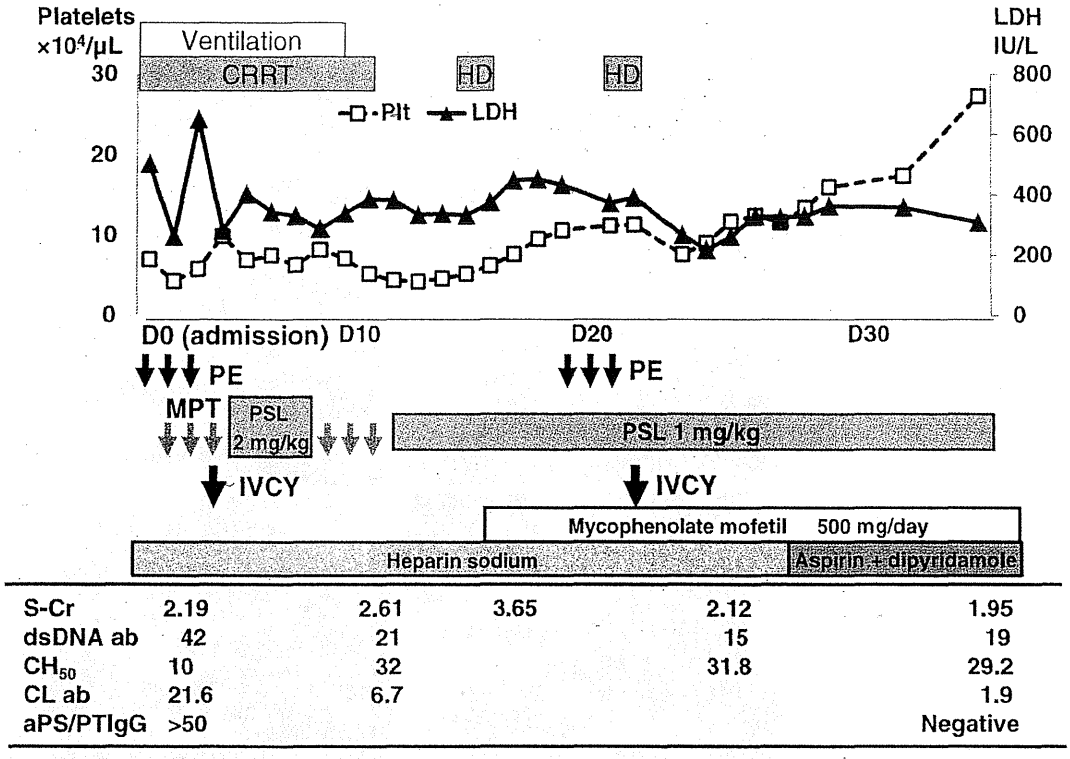

Fig. 1 Clinical course after admission to our institute. The treatment used and laboratory data after admission are shown. *Plt* platelets ($\times 10^4/\mu L$), *s-Cr* serum creatinine (mg/dL), *dsDNA ab* anti-double-strand DNA (IU/mL), *CL ab* anticardiolipin antibody (IU/mL), *aPS/PTIgG* anti-phosphatidylserine–prothrombin complex antibody (U/mL), *PE* plasma exchange, *HD* hemodialysis, *CRRT* continuous renal replacement therapy, *PSL* prednisolone, *MPT* methylprednisolone pulse therapy, *IVCY* intravenous pulse cyclophosphamide, *D* days after admission, *LDH* lactate dehydrogenase, CH_{50} total hemolytic complement activity

or focally sclerotic glomeruli were observed, which is compatible with chronic findings after severe TMA. Approximately 30% of glomeruli were maintained intact. A total of four courses of IVCY were administered. The patient's serum Cr levels were 0.85 mg/dL.

Prompt and aggressive therapies saved the patient and induced complete remission of TMA. Two years after the onset of APS, all APS-related antibodies, including aPS/PT, have remained negative with administration of 4 mg of oral prednisolone and 750 mg of MMF, and anti-platelet (aspirin, dipyridamole) and anti-coagulant (warfarin) treatment. SLE and APS were successfully controlled.

Discussion

Ninety percent of childhood TMA consists of Shiga-like toxin (STx) associated with HUS, and the prognosis is relatively good. TMA, except for STx-related HUS including atypical HUS and TTP, is rare and its prognosis is poor. Congenital or acquired deficiency of complement factor H, factor I, factor B, C3, and membrane cofactor protein, pneumococcal infection, drugs (i.e., ticlopidine, clopidogrel), and congenital defects of ADAMTS13 or its inhibitor (autoantibody) can cause non-STx-related TMA. APS can also lead to development of TMA [10]. Patients with SLE are at increased risk of having TMA due to anti-ADAMTS13 autoantibody [3, 4] or concomitant APS. In addition, survival of patients with TMA, except for those with STx-related HUS, is very poor, especially at younger ages and with concomitant SLE [11].

Our patient showed no decrease in vWF activity and he had negative anti-ADAMTS13 antibody, but was diagnosed with APS because of positive β2GPI, aCA, LA, and aPS/PT. Our patient developed multiple autoantibodies related to APS. Patients with SLE are likely to develop multiple autoantibodies causing TMA, such as anti-ADAMTS13 antibody, anti-antiphospholipid antibody, anticardiolipin antibody, and anti-factor H antibody, which may accelerate endothelial damage and thrombotic events [12, 13]. Additionally, despite early and intensive therapy, mortality is higher in adults who have TTP with SLE compared with patients with idiopathic TTP [14]. Acute renal involvement of APS includes renal artery or vein thrombosis, renal infarction, APS nephropathy (membranous nephropathy), and TMA. Chronic renal involvement is characterized by fibrous intimal hyperplasia

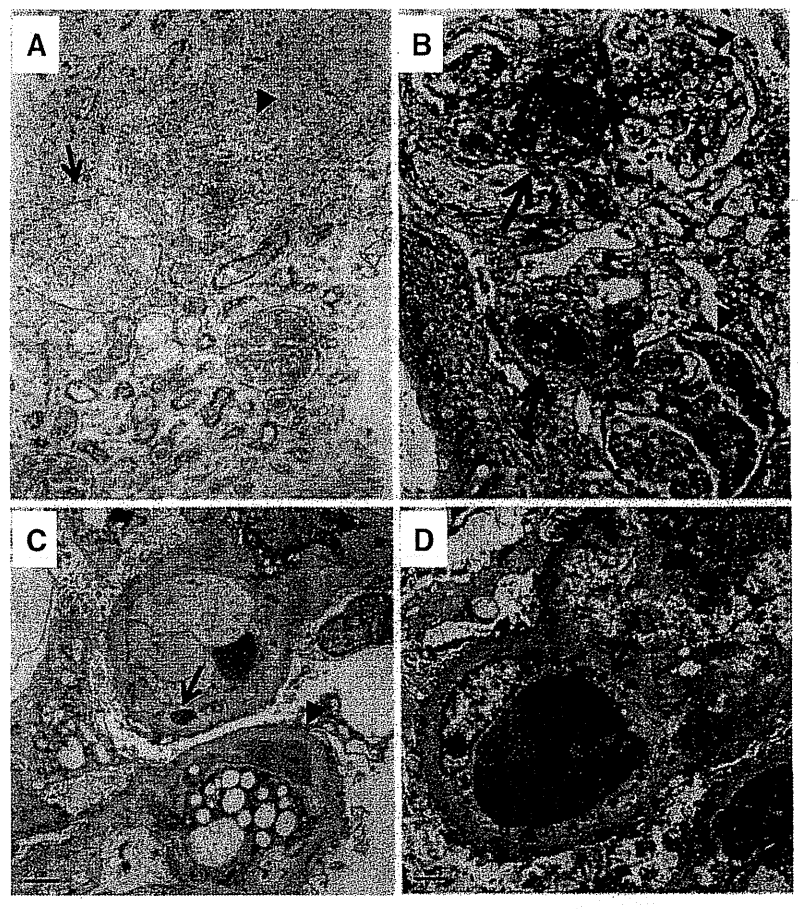

Fig. 2 Renal biopsy at the onset of disease. **a** Diffuse and severe mesangiolysis in a glomerulus (*arrow*) and a ruptured glomerulus (*arrowhead*; PAS stain, ×200). **b** Microvascular thrombi in an afferent artery (*arrows*) and diffuse mesangiolysis in glomeruli (*arrowheads*) can be clearly seen (Masson stain, ×400). **c** Subendothelial expansion (*arrowhead*) and subendothelial deposits (*arrow*) in degenerated endothelium. **d** Intracapillary thrombi (*arrow*)

and focal cortical atrophy [15]. Clinical manifestation of renal involvement of APS presents as arterial hypertension, renal insufficiency, and proteinuria, as experienced in our patient.

The aPS/PT is formed as anti-prothrombin complex antibodies in the presence of phosphatidylserine and calcium, and it is one of the lupus anticoagulants. Although it is not included in the diagnostic criteria, aPS/PT is considered to be a new and specific diagnostic marker of APS. Its sensitivity is 60% and specificity is 96% [8]. Additionally, aPS/PT is known to be a highly pathogenic autoantibody, which induces increased thrombin generation in APS patients and causes recurrent fetal loss or thrombosis in multiple organs [16]. IgG aPS/PT is positive in 39% of primary APS, 47% of SLE with APS, 10% of SLE without APS, and 0% in rheumatoid arthritis, Sjögren's syndrome, and other rheumatic diseases. In adult patients, the frequency of thrombotic events, including arterial or venous thrombosis and fetal loss in patients with IgG/IgM aPS/PT, is similar to that in patients with IgG/IgM aCL [8]. In addition, aPS/PT may cause TMA. Interestingly, two adult patients with SLE/APS, who had a high aPS/PT, but no anticardiolipin antibody, developed TMA [9]. ADAMTS13 activity was normal and anti-ADAMTS13 antibody was not detected in either of the patients. This finding suggests that aPS/PT itself may induce TMA. To the best of our knowledge, we report the first pediatric patient with APS and aPS/PT who developed TMA.

Peyvandi et al. demonstrated that anti-ADAMTS13 antibody is one of the major causes of TTP/TMA, but half of patients with TTP/TMA showed no or a mild decrease in ADMATS13 and absence of autoantibody. Renal impairment and clinical prognosis of ADAMTS13-related TMA is as severe as that of non-ADAMTS13-related TTP/TMA [17]. Non-ADAMTS13-related TTP/TMA could contain APS and complement-related disorders, as described at the beginning of this discussion. In fact, the mortality of TMA associated with APS is 22% [18]. If patients with TMA show normal ADAMTS13 activity, aCL and β2-GPI as well as aPS/PT should be examined. However, unfortunately, aPS/PT is still not broadly and commercially available. Therefore, some patients with TMA may not be correctly diagnosed as having APS. However, 90% of patients with positive aPS/PT have lupus anticoagulant [8]. Therefore, if a patient does not have aCL or β2-GPI, but does have LA, aPS/PT should be taken into consideration.

Although the actual frequency of TMA in patients with SLE is unknown, 1% of lupus patients with SLE develop TMA [15]. In Japan, 27 out of 6392 adult patients with SLE (0.14%) developed TMA (Health and Labour Ministry data

in 2005, published in Japanese). A report from the UK revealed that 7 out of 257 renal biopsy samples showed TMA [19]. The frequency of TMA in childhood lupus may be less than that in adults. However, both lupus nephritis and TMA should be taken into consideration if a patient with lupus develops acute renal injury. Urgent and aggressive intervention is essential for TMA because of its poor renal and survival prognosis [20]. Although we did not initially have results for aPS/PT, ADAMTS13 activity or anti-ADAMTS13 antibody for our patient, we immediately started plasma exchange and aggressive immunosuppressive therapy including IVCY and MMF, and the patient recovered. In acute thrombotic events, patients with APS should be treated with heparin. For chronic management, warfarin is substituted for heparin. In particular, patients who have had venous thrombosis are recommended life-long use of warfarin. Anti-coagulation agents including heparin and warfarin are most beneficial for patients with APS. On the other hand, anti-platelet therapy is of minimal benefit for the prevention of thrombosis in APS.

Our report suggests that not only anti-ADMTS13 antibody, but also APS-related autoantibodies such as β2GPI, aCL, and aPS/PT can cause TMA. The frequency of aPS/PT in pediatric patients with APS is still unknown. A broader distribution of the assay system for aPS/PT is necessary and this may improve the prognosis of TMA due to APS.

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BRIEF REPORT

Atypical *Pneumocystis jiroveci* pneumonia with multiple nodular granulomas after rituximab for refractory nephrotic syndrome

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Abstract
Background Rituximab, an anti-CD20 antibody that targets B cells, is a promising agent against steroid-dependent and steroid-resistant nephrotic syndrome in children.
Case-Diagnosis/Treatment We report a 3-year-old boy who presented with atypical *Pneumocystis jiroveci* pneumonia (PCP) following administration of rituximab for refractory nephrotic syndrome. He had received cyclosporine and daily prednisolone for over 1 year. Following rituximab therapy, a hazy shadow was observed on his chest X-ray. Chest-computed tomography revealed multiple nodular lesions in bilateral lungs, although his clinical symptoms were subtle. PCR analysis demonstrated the presence of *Pneumocystis* DNA in his bronchoalveolar lavage. Lung wedge resection of the nodular lesion exhibited granulomas containing a few cysts of *P. jiroveci* that primarily consisted of T cells and histiocytes and lacked B cells. A deficiency of B cells following rituximab treatment suggests a dramatic effect on the immune response and, therefore, could result in granulomatous PCP. Nodular granulomatous lesions of PCP comprise an emerging concept previously reported in adults with hematological disease, bone marrow transplant, or treatment with rituximab. We report the first pediatric case of nodular PCP. Granulomatous PCP can be life-threatening. Moreover, bronchoalveolar lavage often fails to demonstrate the presence of *P. jiroveci* DNA. Wedge biopsy is warranted for definitive diagnosis. Our patient fully recovered with sulfamethoxazole/trimethoprim treatment because of early detection.
Conclusions The indication of rituximab for refractory nephrotic syndrome has increased recently. Therefore, recognition of the risk of atypical PCP is important. Our findings suggest that PCP prophylaxis should be considered following rituximab therapy.

Keywords Nephrotic syndrome · Rituximab · *Pneumocystis jiroveci* · Granulomatous · Granulomatous *Pneumocystis jiroveci* pneumonia

Abbreviations

PCP	*Pneumocystis jiroveci* pneumonia
RTX	Rituximab
NS	Nephrotic syndrome
FRNS	Frequently relapsing nephrotic syndrome
SDNS	Steroid-dependent nephrotic syndrome
SRNS	Steroid-resistant nephrotic syndrome

Introduction

Treatment of refractory childhood nephrotic syndromes, such as frequently relapsing nephrotic syndrome/steroid-dependent (FRNS/SDNS) and steroid-resistant nephrotic syndrome (SRNS), remains a challenge. Although various

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immunosuppressive agents are effective, a substantial number of children are intractable. Recently, rituximab (RTX), a monoclonal antibody that targets the B cell specific antigen CD20, has been demonstrated to be effective for FRNS/SDNS and SRNS in children [1–3]. RTX is relatively well tolerated; however, occasionally severe or life-threatening adverse events occur, including progressive multifocal leukoencephalopathy [4], interstitial pneumonia [5], and ulcerative colitis [6]. Moreover, a decreased number of B cells potentially induces opportunistic infection and deterioration of infection. *Pneumocystis jiroveci*, formerly known as *Pneumocystis carinii*, pneumonia (PCP) is a rare but serious cause of mortality in patients with acquired immunodeficiency syndrome (AIDS), as well as in immunocompromised hosts. RTX increases susceptibility to PCP. Previous cases of PCP following rituximab treatment have been reported for various indications, such as B cell lymphoma [7–9], rheumatoid arthritis [10], Wegener's granulomatosis [11], autoimmune hemolytic anemia [12, 13], pure red cell aplasia [14], pemphigus [15], and acute rejection of kidney transplants [16, 17]. PCP usually presents as a bilaterally diffuse and fairly symmetric interstitial pattern on chest X-ray and as patchy ground-glass opacities on high-resolution computed tomography (hrCT). However, immunocompromised hosts who exhibit hematological malignancy with or without bone marrow transplants, chemotherapy including RTX, and AIDS infrequently present with atypical PCP with multiple nodular granulomatous lesions [18–20]. We report the first pediatric case of nodular granulomatous PCP in which the patient was treated with a single dose of 375 mg/m^2 of RTX against SDNS. RTX potentially increases the risk of granulomatous PCP. Written informed consent for publication of this information was obtained from the child's family.

Case report

The patient is a 3-year-old boy with SDNS. He had been healthy before he presented with NS at 2 years of age. At primary onset, he was initially treated with 25 mg/day (60 mg/m^2/day) of daily prednisolone (PSL) for 4 weeks. NS was in remission 10 days after initiation of PSL. PSL was then reduced to 16 mg/day (40 mg/m^2) on alternate days, but NS had relapsed soon after PSL reduction. He was treated with 25 mg of daily PSL again. Proteinuria resolved in 7 days. However, as PSL was reduced to 20 mg/day on alternate days, he experienced a second relapse. Thereafter, we stopped reducing PSL by administration on alternate days. Nevertheless, his NS repeatedly relapsed whenever daily PLS was reduced to less than 15 mg. Cyclosporine, losartan and mizoribine were added after 5, 8, and 9 months after diagnosis, respectively. Renal biopsy was performed 5 months after the onset of NS.

One year after onset, at his fifth relapse, the patient was treated with daily PSL 18 mg/day (32 mg/kg/m2), cyclosporine 50 mg/day (3.3 mg/kg/day), and losartan 15 mg/day (1 mg/kg/day). He was then treated with daily PSL 25 mg/day (1.8 mg/kg/day) for 4 weeks. The dose of PSL was slightly less than that used previously, because he was intolerant to the previous full dose of PSL owing to a mood disorder. After 4 weeks, his urinary protein/creatinine ratio was still 6, and serum albumin level was 2.5 g/dl. Additionally, he suffered from serious steroid-related toxicities, including growth retardation, obesity, and bilateral glaucoma. He was transferred to our institute because of SRNS.

After admission, he was treated with one course of methylprednisolone pulse therapy (30 mg/kg/day, three consecutive days). Four days after this therapy, his NS completely remitted. Thereafter, we administered 375 mg/m^2 of RTX 2 days after the remission, and we added two more courses of methylprednisolone pulse therapy for reduction of steroids and prevention of further relapses. The patient did not have a reaction to RTX infusion, and was finally discharged. After RTX infusion, daily PSL 1 mg/kg/day was continued for 1 month, and it was reduced to 0.25 and 0.5 mg/kg/day every other day. Cyclosporine was continued, and its 2-h blood concentration was maintained between 400 and 600 ng/dl. However, his NS relapsed 50 days after RTX infusion, and he was admitted to our clinic again. Upon admission, he experienced no symptoms. His temperature was 36.3 °C, heart rate was 110/min and blood pressure was 100/50 mmHg. However, his respiratory rate was slightly fast (30/min). Oxygen saturation remained above 95 % while the patient was awake, but dropped to 92 % during sleep. Other physical examinations did not exhibit any remarkable findings. However, a chest radiograph exhibited patchy and solid infiltrates spread over both lungs (Fig. 1a). Chest helical computed tomography (CT) revealed multiple solid opacities in bilateral lobes.

A peripheral blood examination showed the following: hemoglobin, 13.0 g/dl; platelets, 34.6×10^4/μl; and white blood cells, 6.1×10^4/μl with 72.9 % neutrophils, 18.2 % lymphocytes, 5.2 % monocytes, and 1.5 % eosinophils. The biochemistry profile indicated normal liver and renal function. Lactate dehydrogenase was elevated to 500 IU/l and the isoenzyme patterns suggested that the origin was the lung. The CRP level was 1.0 mg/dl, and erythrocyte sedimentation rate was 44.0 mm/1 h. Serum creatinine and blood urea nitrogen were 0.15 and 11.6 mg/dl, respectively, and serum total protein and albumin were 5.1 and 3.5 g/dl, respectively. Urinary protein was 208.5 mg/dl, and the urinary protein/creatinine ratio was 2.1. The amount of CD19- and CD20-positive B cells in peripheral blood was zero (before RTX infusion, they were 552 and 604/μl, respectively). β-d-Glucan was elevated to 322 pg/ml (normal: <20 pg/ml). Cytomegalovirus antigenemia, Epstein–Barr virus genome, and QuantiFERON (test for tuberculosis)

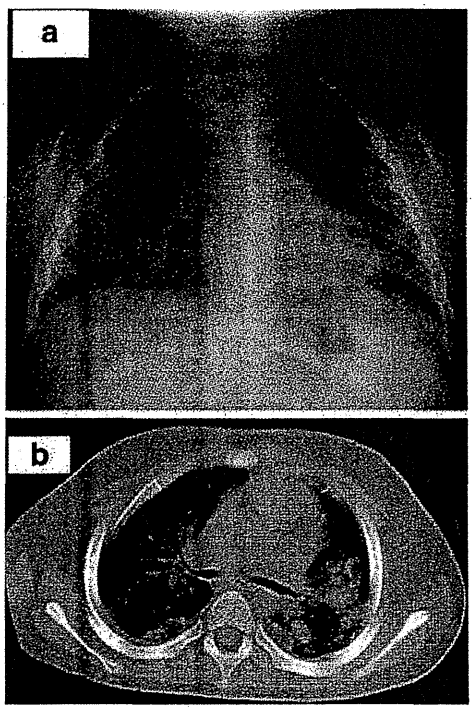

Fig. 1 Chest X-ray and high-resolution computed tomography (CT) scan of the lung. **a** Chest X-ray shows patchy and solid infiltrates spread over both lungs on admission. **b** A high-resolution CT scan of the chest 3 weeks after admission shows exacerbation of solid opacities in both lobes

results were all negative. Urinalysis exhibited mild albuminuria (urinary protein: 208 mg/dl, urinary creatinine: 99 mg/dl). Because of marked elevation of β-d-glucan, infection with fungus or *P. jiroveci* was suspected. We performed bronchoalveolar lavage (BAL) under general anesthesia on day 3. After bronchoscopy, the patient was treated with oral TMP-SMX for *Pneumocystis* at a dose of TMP 5 mg/kg every 6 h and voriconazole for fungus, including *Aspergillus* 15 mg/kg/day. PSL was maintained at 10 mg/day to prevent a withdrawal reaction, and cyclosporine was halted. Microscopy was negative but polymerase chain reaction analysis revealed *Pneumocystis* DNA. No other pathogens were detected in his blood culture and BAL. We stopped voriconazole and continued a full dose of TMP-SMX for 3 weeks followed by a prophylactic dose of TMP (5 mg/kg/day). Although CD19- and CD20-positive cells in peripheral blood recovered 8 months after RTX, he was treated with prophylactic TMP for 10 months because he was still being treated with MMF.

Although the patient's oxygen saturation improved, and β-d-glucan returned to a normal range, lactate dehydrogenase levels remained elevated. Another chest hrCT scan 3 weeks from the initiation of TMP-SMX demonstrated a further increase in the number and size of the pulmonary nodules (Fig. 1b). To rule out any other causes that were overlooked, the patient underwent open thoracic wedge resection of the nodule lesion. A biopsy sample showed necrotic granulomas containing a few cysts of *P. jiroveci* and Ziehl-Neelsen staining was negative (Fig. 2a/e). Immunohistochemistry revealed that the granulomas primarily consisted of CD3-positive T cells and CD68-positive histiocytes; however, there was an absence of B cells (Fig. 2b, c, d). His respiratory status remained stable, and lactate dehydrogenase soon returned to the normal range. A chest hrCT on the 52nd day exhibited an apparent decrease in nodular lesions. The patient's NS also spontaneously remitted along with the recovery of pulmonary findings.

Discussion

This is the first childhood case of granulomatous PCP. Additionally, this is the first case of PCP following treatment with rituximab against SDNS. PCP is a serious cause of mortality in immunocompromised patients, affecting up to 50 % of patients. Our patient was treated with a high dose of daily PSL, cyclosporine, and mizoribine for an extended period. Additional rituximab potentially strengthens immunosuppression and increases the susceptibility to PCP. However, our patient's clinical and radiological findings substantially differed from typical PCP, and we had to rule out viral or fungal infection, tuberculosis, and rituximab-induced interstitial pneumonia.

Accumulating evidence suggests that, under some circumstances, RTX may significantly increase the risk of PCP. Recently, cyclophosphamide, doxorubicin, vincristine and prednisone with rituximab (R-CHOP) against B-cell lymphoma was shown to increase the prevalence of PCP compared with classical CHOP (identical treatment without RTX). Additionally, PCP after rituximab has been previously reported in patients with rheumatoid arthritis, Wegener's granulomatosis, autoimmune hemolytic anemia, pure red cell aplasia, pemphigus, and acute rejection of kidney transplants. Therefore, B cell depletion by RTX may increase the risk of PCP. They suggested the necessity of PCP prophylaxis after RTX therapy.

The host's defense against *Pneumocystis* is thought to be critically dependent on CD4+ helper T cells. The incidence of PCP in human immunodeficiency virus (HIV) infected patients is elevated when the level of circulating CD4 cells falls below 200/μl. However, B cells potentially play a significant role in protection against *Pneumocystis*. Transgenic B-cell functionally deficient mice, in which B cells do not express major histocompatibility complex class 2 antigens (and thus were unable to act as antigen-presenting cells), are susceptible to *Pneumocystis carinii* (formerly the species *Murina*). These mice fail to clear the *Pneumocystis* infection, most likely because of the inefficient generation of protective CD4+ memory and effector T cells in the lungs [21]. The

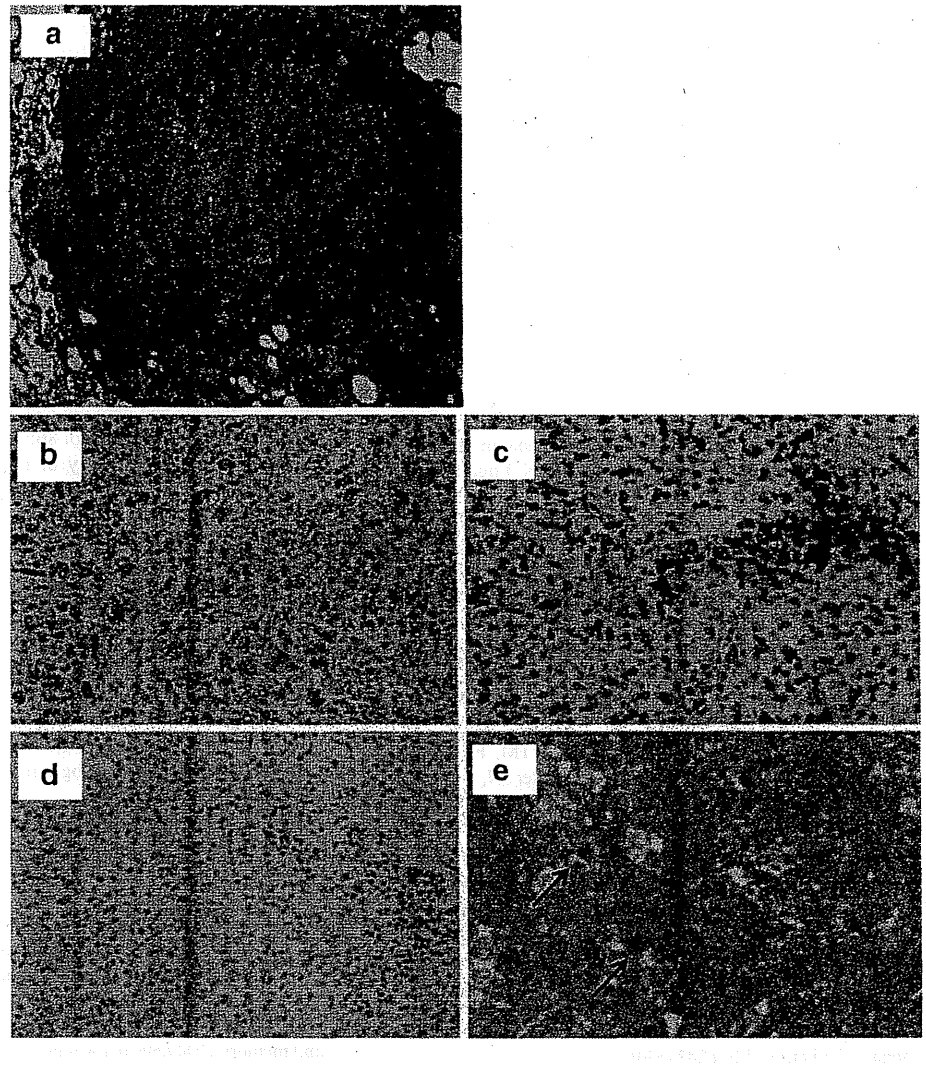

Fig. 2 Pathological findings of lung biopsy. The biopsy sample shows necrotic granulomas (**a**, hematoxylin-eosin stain, magnification ×100), consistent with CD3-positive T cells (**b**) and CD68-positive histiocytes (**c**) (positive cells were stained brown), but without CD20-positive B cells (**d**). Several cysts of *P. jiroveci* were identified inside of the granulomas by Grocott's stain (**e**, *arrows*)

deficiency of B cells potentially results in attenuated immunoprotection against *P. jiroveci*.

Our patient was likely infected with *P. jiroveci*. However, he developed granulomatous PCP after RTX treatment; therefore, RTX potentially contributed to the development of granulomatous PCP. A defect in B cells induces high susceptibility to *P. jiroveci*, as well as development of granulomatous PCP. Granulomatous PCP is an emerging concept. Granulomatous PCP has been previously reported in up to 4–5 % of PCP patients. A total of 35 adult patients have previously exhibited granulomatous PCP. They suffered from AIDS, hematological neoplasms, and solid malignancy, but none of these patients had NS. Interestingly, three of them were treated with RTX prior to the development of PCP [19, 20]. B cell function appears to be impaired in most of these patients, as in our patient. In our patient, immunohistochemical staining revealed that granulomas primarily consisted of T cells and histiocytes without B cells. B cells may be essential for the clearance of *P. jiroveci*, and macrophages may substitute for the clearance of *P. jiroveci* by forming granulomas. In fact, we found several cysts of *P. jiroveci* inside the granulomas. B cell deficiency following RTX potentially modifies the immune response to *P. jiroveci* and induces a granulomatous reaction. Furthermore, Totet et al. reported that granuloma formation is not related to any specific genotype of *P. jiroveci* [22, 23].

Although our patient exhibited no symptoms and his general condition was well on admission, the mortality rate can be 35–50 % in patients without HIV compared with 10–20 % in those with HIV [24, 25]. The diagnosis of conventional PCP is traditionally performed by BAL. However, in the case of granulomatous PCP, BAL frequently fails to detect *P. jiroveci* [18]. An open lung biopsy is required to generate the correct diagnosis if BAL fails to detect *P. jiroveci*.

Our case study suggests that RTX may modulate T cell immunity and increase susceptibility to PCP. Furthermore, RTX treatment may cause a granulomatous pattern and make the correct diagnosis of PCP difficult. Indication of rituximab

for refractory NS has been recently expanding. Therefore, physicians should be aware of the risk of PCP and its atypical manifestations under combined immunosuppressive therapy, including RTX. Prophylaxis for PCP should be considered after RTX against refractory NS.

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Pediatric liver–kidney transplantation for hepatorenal fibrocystic disease from a living donor

Sakamoto S, Kasahara M, Fukuda A, Tanaka H, Kakiuchi T, Karaki C, Kanazawa H, Kamei K, Ito S, Nakazawa A. Pediatric liver–kidney transplantation for hepatorenal fibrocystic disease from a living donor. Pediatr Transplantation 2012: 16: 99–102. © 2011 John Wiley & Sons A/S.

Abstract: The indications for and the timing of LT and/or KT for the patients with HRFCD are based on the severity of liver and kidney involvement. Most organs come from living donors, because the number of deceased donors is extremely low in Japan. Therefore, patients with HRFCD may need two organs from living donors. Four patients with HRFCD underwent living donor LT and KT from a single donor. The type of transplantation included combined LKT in one case, sequential LKT in two cases, and sequential KLT in one case. Although the case of combined LKT died because of sepsis, the other cases were doing well. Sequential LKT was successfully performed at the proper timing for each transplant; however, both of the donors suffered from a gastroduodenal ulcer after liver donation because of the psychological burden related to the relatively short period between two donations. In conclusion, living donation for LKT with cautious surgical procedures is not harmful for donors and recipients. However, changes in the allocation system established for deceased donors for HRFCD should be considered to avoid the need for two organ donations from the same living donor.

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Key words: hepatorenal fibrocystic disease – living donor – liver and kidney transplantation

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Caroli's disease and CHF are often associated with ARPKD, characterized as HRFCD (1). The concomitant renal insufficiency may lead to a poor prognosis for the patients undergoing LT, although apheresis therapies have been safely introduced even for low-weight pediatric patients (2). At present, the indications for and the timing of LT with KT for the patients with HRFCD are unclear. This report describes our recent experience with living donor LKT from a single donor in four patients with HRFCD.

Abbreviations: ARPKD, autosomal recessive polycystic kidney disease; CAPD, continuous ambulatory peritoneal dialysis; CCr, creatinine clearance; CHF, congenital hepatic fibrosis; Cys-C, cystatin C; ESRD, end-stage renal disease; GFR, glomerular filtration rate; HRFCD, hepatorenal fibrocystic disease; KLT, kidney–liver transplantation; KT, kidney transplantation; LDLT, living donor liver transplantation; LKT, liver–kidney transplantation; LT, liver transplantation; SRTR, Scientific Registry of Transplant Recipients.

Patients and methods

Between 2005 and 2010, 154 children underwent LDLT with an overall patient survival of 92.0%. Four patients with HRFCD were indicated for LDLT because of repeated cholangitis in Caroli's disease (n = 2) and portal hypertension in CHF (n = 2). The kidney disease consisted of ARPKD in three cases, including one post-KT case, and nephronophthisis in one case.

LDLT was performed in a piggyback fashion without a veno-veno bypass, as described elsewhere (3). The immunosuppression consisted of tacrolimus and low-dose steroids. Mycophenolate mofetil was initiated as the third drug for renal-sparing immunosuppression.

During the follow-up period, the assessment of the renal function continued to be performed by nephrologists. The renal function was evaluated by determining such parameters, as the serum CCr, GFR, and Cys-C levels. Once the renal function exhibited a clear declining trend, KT was subsequently performed. After KT, the immunosuppression

consisted of the same tacrolimus-based regimen for pediatric KT as described elsewhere (4).

All of the donors consulted psychologists before donations as a mandatory screening to confirm their willingness to donate their organs, and they received psychological management after donations, if necessary.

Results

The characteristics of the recipients and donors are summarized in Table 1. Combined LKT was performed in one case (case 1), sequential LKT in two cases (cases 2 and 4), and sequential KLT in one case (case 3). The chronological changes in renal function in sequential LKT cases are summarized in Table 2.

Case 1

A four-yr-old girl with ARPKD had been on CAPD since the age of seven months, and she underwent bilateral nephrectomy because of renal enlargement. Caroli's disease was diagnosed by a liver biopsy at the age of one yr. She had experienced repeated cholangitis with several episodes of sepsis, and she was referred to the hospital for LKT. Her father willingly donated his left lateral segment and his right kidney simultaneously. The liver and kidney allografts functioned well after her transplant without hemodialysis, septic shock suddenly occurred two wk after her transplant with a positive finding of Pseudomonas species in a blood culture, and immunosuppression had to be withdrawn. Her liver and kidney functions thereafter both deteriorated because of rejection, and she died of multi-organ failure because of sepsis two months after undergoing transplantation.

Case 2

A five-yr-old boy with CHF, which was diagnosed by a liver biopsy at the age of two yr, had been followed at a local hospital because of renal dysfunction. Ascites retention gradually increased because of portal hypertension at the age of five yr, which was responsive to medical treatment. He was referred for LKT. Although his liver dysfunction was mild, there were severe clinical findings related to portal hypertension, such as pancytopenia, esophageal varices, and massive ascites retention. His renal function had gradually deteriorated, CCr, 34.8 mL/min/1.73 m^2, but dialysis was not indicated. An isolated LDLT was first scheduled, and the timing of KT was dependent on his renal function after LDLT. He received a left lateral segment from his father. His liver functioned well; however, ascites retention still remained after LDLT. His renal function gradually worsened (Table 2), and hemodialysis was initiated. KT was performed four months after LDLT. His father donated his left kidney. His postoperative course after KT was uneventful, except for the progression of anemia possibly related to anti-donor antibody, which was successfully treated by steroid pulse therapy.

Case 3

A six-yr-old boy with ARPKD had been on CAPD since the age of five months and underwent bilateral nephrectomy because of renal enlargement. He underwent KT at the age of three yr, by receiving a kidney graft from his father. His postoperative course was uneventful without any episode of rejection. Hepatomegaly was detected at the outpatient clinic at six yr of age, and he presented with bleeding from esophageal varices, which were successfully treated by endoscopic variceal ligation. He was referred for LT. His laboratory data showed severe thrombocytopenia owing to hypersplenism. His renal allograft was functioning well under minimal immunosuppression with tacrolimus, mizoribine, and low-dose steroids. He received a left lateral segment from his father. His postoperative course was uneventful.

Case 4

A seven-yr-old girl was followed because of renal dysfunction since the age of four months at a local hospital. Bile duct dilatation was detected

Table 1. Clinical characteristics of the recipients and donors

Case No.	Age at LT	Gender	Original disease: Liver/kidney	Donor	Age at LD	ABO compatibility	Type of transplantation	Interval between transplants	Follow-up period	Outcome
1	4 yr 3 months	F	Caroli's disease/ARPKD	Father	33 yr	Incompatible	Combined LKT	–	2 months	Died (sepsis)
2	5 yr 5 months	M	CHF/nephronophthisis	Father	45 yr	Compatible	Sequential LKT	4 months	1 yr 6 months	Alive
3	6 yr 10 months	M	CHF/ARPKD	Father	47 yr	Compatible	Sequential KLT	3 yr 4 months	4 yr	Alive
4	7 yr 5 months	F	Caroli's disease/ARPKD	Father	40 yr	Compatible	Sequential LKT	4 months	7 months	Alive

ARPKD, autosomal recessive polycystic kidney disease; CHF, congenital hepatic fibrosis; KLT, kidney–liver transplantation; LD, liver donation; LKT, liver–kidney transplantation; LT, liver transplantation.

Table 2. Chronological changes in the renal function parameters in sequential LKT

Case No.	Serum Cr (mg/dL)					CCr or GFR (mL/min/1.73 m^2)*					Cys-C (mg/L)				
		After LT		After KT			After LT		After KT			After LT		After KT	
	Before LT	1 M	3 M	1 M	3 M	Before LT	1 M	3 M	1 M	3 M	Before LT	1 M	3 M	1 M	3 M
2	1.12	2.58	3.09	0.36	0.22	34.8	4.5	N/A	97.4	140.9	2.68	4.40	4.85	0.55	0.88
4	1.71	1.86	2.12	0.34	0.31	32.2	N/A	18.8	117.5	129.5	2.59	N/A	2.81	0.75	0.73

Cr, creatinine; CCr, creatinine clearance; Cys-C, cystatin C; GFR, glomerular filtration rate; KT, kidney transplantation; LT, liver transplantation; LKT, liver and kidney transplantation; N/A, not applicable.
*CCr was followed as a renal function parameter in case 2, and GFR was followed in case 4.

during an examination for a persistent fever of unknown origin at the age of six months, and she underwent hepaticojejunostomy based on a diagnosis of congenital biliary dilatation. She experienced repeated episodes of cholangitis, and she was referred for LKT. Her GFR, calculated by inulin clearance, was 32.2 mL/min/1.73 m^2 at admission. The isolated LDLT was scheduled first, and the timing of KT would be determined by her renal function after LDLT. Her liver functioned well without any episodes of rejection. Her renal function gradually worsened (Table 2). KT was performed four months after LDLT. Her father donated his left kidney. Her postoperative course after KT was uneventful.

Donors

All of the donors donated a part of the liver and a kidney simultaneously or sequentially, because there was no other candidate for donation among the patients' family members. There were no surgical complications related to the donations in any of the donors; however, two donors, both of whom were donors for sequential LKT, developed gastroduodenal ulcers after the liver donation.

Discussion

Previous reports have discussed the indications for and the timing of LT and/or KT for patients with HRFCD, although these issues are still controversial (5, 6). Liver disease is chronic with preserved synthetic function in most patients with HRFCD; thus, isolated KT is usually employed in the setting of ESRD; however, the presence of hepatic disease in patients with HRFCD who undergo isolated KT can adversely impact outcomes (7). The previous retrospective study of isolated KT for ESRD with HRFCD showed a higher incidence of sepsis-related mortality, which might reflect an increase incidence of bacterial infection of the biliary tree related to the liver involvement of HRFCD (8). Combined LKT should therefore be considered when progressive and severe complications owing to liver disease occur (8). The outcome of combined LKT has improved. The SRTR data show that long-term prognosis is good with a five-yr survival rate of 75.5% (9). However, it is still lower than that of single-organ transplantation, depending on the primary disease and the patient's condition at LKT (10). As shown in case 1 in this current series, combined LKT in patients with a profoundly ill status leads to more difficult postoperative management, which may lead to fatal complications, especially sepsis

originating in the biliary tree. Postoperative complications and nephrotoxic immunosuppressants can lead to a progressive decline in renal function after LT, and they may result in hemodialysis dependence during perioperative management. Hemodialysis after pediatric LT is difficult to perform because of the presence of an unstable hemodynamic state and a tendency for bleeding (2). Ueno et al. (11) reported a greater risk for significant post-transplant deterioration of renal function, almost 50% reduction in GFR, in patients with HRFCD undergoing isolated LT, even though all patients appeared to have reasonable renal function prior to LT. Recent remarkable advances in LT have yielded survival for pediatric recipients. Therefore, LT should be performed before renal insufficiency becomes far-advanced to avoid missing the proper timing. Even though sequential KT has to be considered when there is progression of renal insufficiency after LT, the recovery of liver function provides advantages for the successful outcome of this procedure (2).

The concept of an LDLT program should be established, considering the donor safety as the first priority. Two donors in this series suffered from a gastroduodenal ulcer after liver donation. On the other hand, the incidence of a gastroduodenal ulcer in the donors undergoing only liver donation was 2.0%, three of 150 donors at our institute. This indicates that not only surgical stress but also the psychological burden may negatively affect this complication because of the relatively short period between two donations. As a result, psychological management of a living donor who has the possibility to undergo two donations is crucial for a successful LDLT program.

In Japan, the number of deceased donors is still extremely low, especially for pediatric recipients. Our allocation policy for liver and kidney organs accords a liver recipient priority for receiving a kidney from the same deceased donor. The majority of patients with HRFCD may require combined or sequential LKT, although the disease progression of both of the organs is not parallel. Given the shortage of cadaveric organs, it is often unavoidable to use a living donor for at least one organ; however, to avoid two organ donations from the same living donor, our allocation policy for liver and kidney organs should be modified to accord the HRFCD recipients, who have already undergone their first organ transplantation from a living donor, priority to receive the other organ.

In conclusion, our present experience demonstrated that living donation for LKT with cautious surgical procedures is not harmful for donors and recipients even after a long-term follow-up. However, changes in the allocation system of the deceased donors for HRFCD should be considered to avoid the need for two organ donations from the same living donor.

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Authors contribution

S.S. and M.K.: study design, data analysis, writing of the paper; T.S., A.F., T.K., C.K., H.K. and K.K.: data analysis; H.T.: study design; S.I. and A.N.: study design, data analysis.

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Focal Segmental Glomerulosclerosis in Patients With Complete Deletion of One *WT1* Allele

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KEY WORDS

deletion, focal segmental glomerulosclerosis, WAGR syndrome, *WT1*

ABBREVIATIONS

ACEI—angiotensin-converting enzyme inhibitor
BUN—blood urea nitrogen
CrCl—creatinine clearance
DDS—Denys-Drash syndrome
DMS—diffuse mesangial sclerosis
FSGS—focal segmental glomerulosclerosis
WAGR—Wilms' tumor, aniridia, genitourinary anomalies, and mental retardation

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abstract

The renal prognosis of patients with Wilms' tumor, aniridia, genitourinary anomalies, and mental retardation syndrome (WAGR) is poor. However, the renal histology and its mechanisms are not well understood. We performed renal biopsies in 3 patients with WAGR syndrome who had heavy proteinuria. The complete deletion of one *WT1* allele was detected in each patient by constitutional chromosomal deletion at 11p13 using G-banding, high-resolution G-banding, and fluorescence in situ hybridization. The patients exhibited proteinuria at the ages of 6, 10, and 6 years and were diagnosed as having focal segmental glomerulosclerosis (FSGS) at the ages of 7, 16 and 19 years, respectively. They exhibited normal or mildly declined renal function at the time of biopsy. Re-examination of a nephrectomized kidney from 1 patient revealed that some glomeruli showed segmental sclerosis, although he did not have proteinuria at the time of nephrectomy. The other 2 patients did not develop Wilms' tumor and thus did not undergo nephrectomy, chemotherapy, or radiotherapy, thereby eliminating any effect of these therapies on the renal histology. In conclusion, complete deletion of one *WT1* allele may induce the development of FSGS. Our findings suggest that haploinsufficiency of the *WT1* could be responsible for the development of FSGS. *Pediatrics* 2012;129:e1621–e1625

Miller et al[1] first described WAGR syndrome (Wilms' tumor, aniridia, genitourinary anomalies, and mental retardation). Children with WAGR syndrome invariably have a constitutional chromosomal deletion at 11p13, the region where the *WT1* gene is located. Patients with Denys-Drash syndrome (DDS) usually have a germline missense mutation, which is predicted to result in an amino acid substitution in the eighth or ninth exon of *WT1*. Little et al[2] suggested that the severe nephropathy associated with DDS, which frequently leads to early renal failure, might result from the dominant-negative action of altered *WT1*. By contrast, because of the less severe genital anomalies and apparent lack of nephropathy associated with WAGR, a reduced *WT1* dosage during embryogenesis is thought to have a less pronounced effect on development, especially on renal system development.[3] Breslow et al[4] reviewed nearly 6000 patients enrolled in 4 clinical trials administered by the US National Wilms Tumor Study Group between 1969 and 1995. Of 22 patients with DDS, 13 (59%) developed renal failure; of 46 patients with WAGR, 10 (22%) developed renal failure. The cumulative risks of renal failure at 20 years were 62% and 38%, respectively. These findings suggest that nephropathy is not uniquely associated with missense mutations in *WT1* and that patients with the WAGR syndrome should be followed up closely throughout life for signs of nephropathy.

The renal prognosis of patients with WAGR is poor. However, the renal histology and its mechanisms are not well understood. We therefore performed renal biopsies to reveal the renal pathology in 3 patients with WAGR syndrome who had heavy proteinuria.

CASE REPORTS

Patient 1

Patient 1 was a male diagnosed with bilateral microphthalmos at 1 month of age. Wilms' tumor developed bilaterally at 3 years of age. He also had undescended testes and mental retardation. Previous analysis of G-banded metaphase chromosomes revealed a deletion of chromosome 11p13-15.1 in one allele[5]; the diagnosis of atypical WAGR syndrome was therefore made.[6] Because of a large tumor in the right kidney after the first chemotherapy treatment, the right kidney was nephrectomized. A diagnosis of nephroblastoma (nephroblastic type) was made. At the same time, the contralateral left kidney was biopsied, but no tumor was detected. The nephrectomized kidney revealed that there were no immature glomeruli, and a few glomeruli showed segmental sclerosis (Fig 1 A and B). The patient did not have proteinuria at the time of nephrectomy although microalbuminuria could have been detected.

The patient then underwent a second session of chemotherapy and radiotherapy treatment with left kidney protection. He developed heavy proteinuria at 6 years of age. The left kidney was biopsied (open biopsy) at age 7 years. Renal biopsy findings were consistent with focal segmental glomerulosclerosis (FSGS) (Fig 1 C and D). At the time of biopsy, the patient's height was 107.3 cm (–2.9 SD), weight was 21.7 kg (–0.7 SD), and blood pressure was 120/80 mm Hg. Biochemical data were as follows: total protein, 6.5 g/dL; albumin, 3.3 g/dL; blood urea nitrogen (BUN), 12.9 mg/dL; creatinine, 0.43 mg/dL; 24-hour creatinine clearance (CrCl), 72.2 mL/min/1.73 m^2; early morning urinary protein, 3+ (as measured by using a dipstick test); urinary protein to urinary creatinine ratio, 3.6 (milligram/milligram); and urinary β-2 microglobulin, 0.44 mg/dL (normal range: <0.23 mg/dL). His renal function gradually deteriorated despite angiotensin-converting enzyme inhibitor (ACEI) treatment. At 14 years of age, he underwent a preemptive living-related renal transplantation from his father.

Patient 2

Patient 2 was a male with aniridia, bilateral undescended testes, hypospadias, grade III to IV bilateral vesicoureteral reflux, and mental retardation. High-resolution G-banding revealed deletion of chromosome 11p13-p14.2 in one allele (Fig 2A), and fluorescence in situ hybridization showed heterozygous deletions of *PAX6*, *D11S2163*, *PER*, and *WT1* (Fig 2B), indicating WAGR syndrome. He had a single febrile urinary tract infection at 2 years of age and underwent an antireflux operation at 4 years of age, which resolved his vesicoureteral reflux. A dimercaptosuccinic acid radionuclide scan showed several defects in his right kidney. His proteinuria was detected at 10 years of age by the school urinary screening program. His proteinuria gradually increased, and he underwent renal biopsy (right kidney) at age 16 years. Renal biopsy findings were consistent with FSGS (Fig 1 E and F). At the time of biopsy, the patient's height was 169.2 cm, weight was 67.4 kg, and blood pressure was 128/78 mm Hg. Biochemical data were as follows: total protein, 6.8 g/dL; albumin, 4.3 g/dL; BUN, 25.0 mg/dL; creatinine, 1.20 mg/dL; 24-hour CrCl, 91.0 mL/min/1.73 m^2; early morning urinary protein, 3+ (as measured by using a dipstick test); urinary protein to urinary creatinine ratio, 2.7 (milligram/milligram); daily urinary protein, 3.1 g; and urinary β-2 microglobulin, 0.064 mg/dL. At the latest follow-up (24 years of age), his renal function was stable (BUN: 25.0 mg/dL; creatinine: 1.20 mg/dL) with ACEI treatment, and he had not developed Wilms' tumor.

Patient 3

Patient 3 was a female with aniridia and mental retardation. G-banding revealed deletion of chromosome 11p13-p14 in one allele (Fig 2C), and she was therefore diagnosed with WAGR syndrome. The patient developed proteinuria at

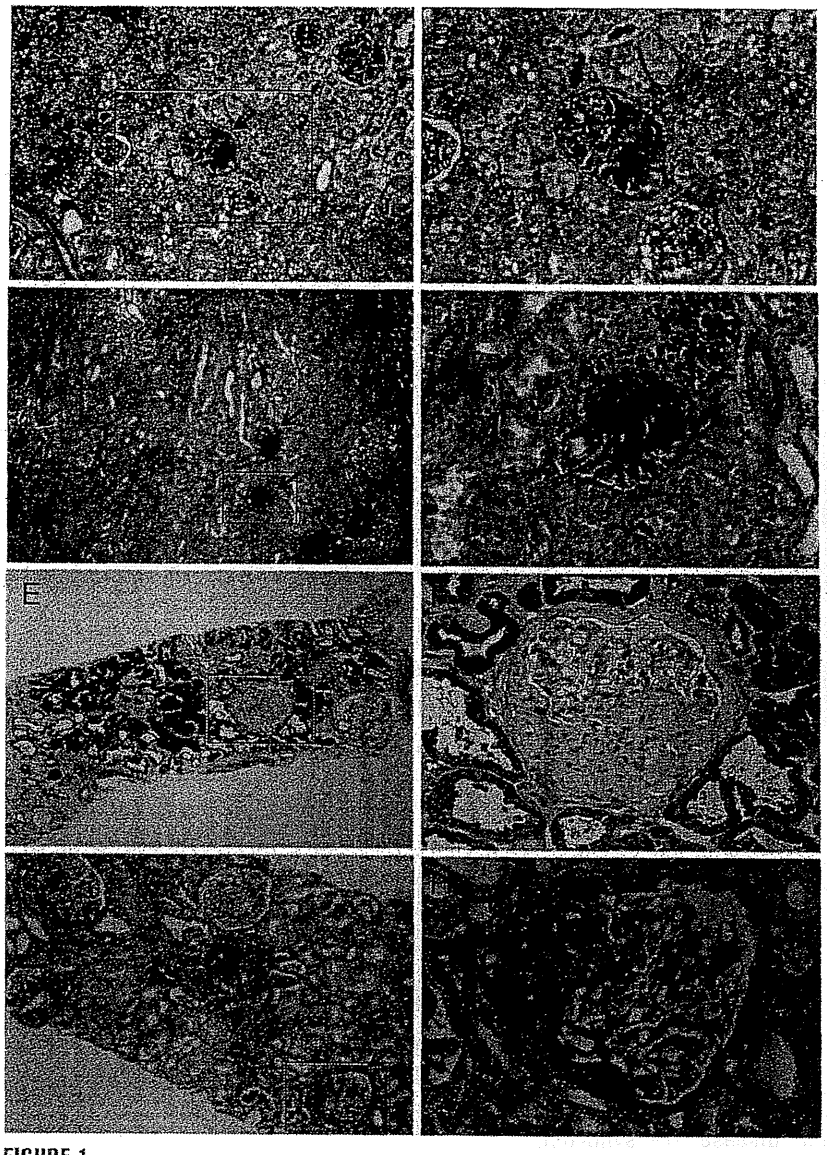

FIGURE 1

Renal histology. A, C, E, and G, Low magnification. B, D, F, and H, High magnification. Arrows show glomeruli with segmental glomerulosclerosis. A and B, Nephrectomized right kidney from patient 1. Patient 1 had no proteinuria at the time of nephrectomy. However, a few glomeruli exhibited segmental glomerulosclerosis although there were no immature glomeruli. C and D, Renal biopsy of left kidney from patient 1. Twenty-eight of 50 glomeruli showed segmental glomerulosclerosis. There were no tubulointerstitial lesions. E and F, Renal biopsy from patient 2. Two of eight glomeruli showed segmental glomerulosclerosis with interstitial fibrosis. G and H, Renal biopsy from patient 3. Ten of 30 glomeruli showed segmental glomerulosclerosis with interstitial fibrosis. All 3 patients exhibited FSGS (not otherwise specified).

the age of 6 years and nephrotic syndrome with normal renal function at age 15 years (urinary protein to urinary creatinine ratio, 10.6 [milligram/milligram]; total protein, 5.6 g/dL; albumin, 2.3 g/dL; BUN, 15.0 mg/dL; creatinine, 0.65 mg/dL; estimated glomerular filtration rate, 100.7 mL/min/1.73 m^2). We were unable to obtain her parents' consent for renal biopsy, and they chose to start drug treatment. However, treatment with prednisolone and ACEI was not effective, and her renal function gradually deteriorated. Therefore, she underwent renal biopsy at age 19 years. At the time of biopsy, her height was 144.5 cm, weight was 72.5 kg, and blood pressure was 130/83 mm Hg. Biochemical data were as follows: total protein, 5.5 g/dL; albumin, 2.5 g/dL; BUN, 30.0 mg/dL; creatinine, 1.40 mg/dL; 24-hour CrCl, 44.65 mL/min/1.73 m^2; early morning urinary protein, 3+ (as measured by using a dipstick test); daily urinary protein, 5.89 g; and urinary β-2 microglobulin, 0.495 mg/dL. Renal biopsy findings were consistent with FSGS (Fig 1 G and H). To date, she has not developed Wilms' tumor.

DISCUSSION

The current study demonstrated that 3 patients with atypical WAGR syndrome developed heavy proteinuria with FSGS, suggesting that the nephropathy seen in this syndrome is responsible for the FSGS lesion.

Patient 1 had possible bilateral Wilms' tumor and underwent unilateral nephrectomy, chemotherapy, and radiotherapy. Therefore, it is possible that the treatment of the remaining kidney for bilateral tumor or nephrogenic rest might account for the development of FSGS. However, the kidney nephrectomized after the first chemotherapy session but before radiotherapy treatment already showed segmental sclerosis in a few glomeruli, suggesting that radiotherapy was not the main cause of FSGS. Chemotherapeutic drugs such as adriamycin may induce FSGS as well as tubulointerstitial inflammation and fibrosis.[7] However, there were no tubulointerstitial lesions, suggesting that chemotherapy might not have been the main cause of FSGS. Nevertheless, it is possible that surgical renal ablation caused FSGS in patient 1.

Patients 2 and 3 did not develop Wilms' tumor during the course of clinical observation, and thus they did not undergo nephrectomy, chemotherapy, or radiotherapy, thereby eliminating any effect of these therapies on renal