An 11-year-old girl with antineutrophil cytoplasmic antibody-associated glomerulonephritis identified by a school urinary screening program

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Abstract Antineutrophil cytoplasmic autoantibody-associated glomerulonephritis (GN) in childhood is rare and has a poor prognosis. We report an 11-year-old girl with renal-limited antineutrophil cytoplasmic autoantibody-associated vasculitis. Proteinuria and hematuria were first detected by a school urinary screening (SUS) program. Histopathological examination revealed pauci-immune necrotizing GN. She did not display purpura or peripheral neuropathy. She was diagnosed with antineutrophil cytoplasmic autoantibody-associated GN based on proteinuria, high serum titers of antineutrophil cytoplasmic autoantibodies (ANCA), and pauci-immune necrotizing GN. The patient was treated with combination therapy, consisting of methylprednisolone and urokinase pulse, prednisolone, mizoribine (MZB), warfarin, and dilazep hydrochloride. At 2 months after treatment, urinary protein excretion was decreased and the hematuria had disappeared, while the serum titer of ANCA was also decreased. The dose of prednisolone was tapered, and proteinuria and hematuria later disappeared at 9 months after treatment. In conclusion, we reported an 11-year-old girl with renal-limited antineutrophil cytoplasmic autoantibody-associated vasculitis early identified by a SUS program and treated with multi-drug combination therapy including MZB. On the basis of our results, we believe that a SUS program may be effective for the early identification and treatment of children with renal-limited antineutrophil cytoplasmic autoantibody-associated vasculitis.

Keywords ANCA-associated glomerulonephritis · School urinary screening · Mizoribine · MPO-ANCA · Child

Introduction

Antineutrophil cytoplasmic autoantibodies (ANCA) constitute a family of autoantibodies directed against various components of the neutrophil cytoplasm. ANCA were first reported by Davies in 1982 [1] in patients with pauci-immune necrotizing glomerulonephritis (GN) with crescents and are now regarded as a serologic marker for active pauci-immune necrotizing crescentic GN, either in the renally limited form or associated with systemic vasculitis, such as granulomatosis with polyangiitis, microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis [2–6].

Although ANCA-associated GN is rare in childhood, there are a number of reviews of the clinical features and outcomes of pediatric ANCA-associated GN [2–5]. Hattori et al. [2] reported that the clinical disease spectrum of ANCA-associated GN is similar in pediatric and adult patients and found that 29 % of patients with ANCA-associated GN progressed to end-stage renal disease (ESRD). Thus, it is important to identify patients with ANCA-associated GN and to manage treatment in the early stages of the disease.

In Japan, a school urinary screening (SUS) program was established in 1974, and a relationship between this program and the early detection of insidious renal diseases has been reported [8, 9]. However, there have been no case reports providing a detailed clinical course for any patient with ANCA-associated GN identified by the SUS program. Herein, we present the case of an 11-year-old girl with

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ANCA-associated GN identified by SUS and treated with multi-drug combination therapy including mizoribine (MZB).

Case report

The patient, an 11-year-old girl, did not demonstrate any thyroid or vascular purpuric disease. Proteinuria and hematuria were first detected by SUS in the spring of 2012. The patient consulted her family doctor and was subsequently referred and admitted to our hospital in June 2012. She had proteinuria and microhematuria, but no hypertension or pretibial edema. Thereafter, the patient showed an increase in proteinuria and macrohematuria after an upper respiratory tract infection in July 2012. She was subsequently admitted to our hospital in August 2012. She did not demonstrate purpura or peripheral neuropathy. Her weight was 40.2 kg, height was 157 cm, and systolic and diastolic blood pressures were 115 and 66 mmHg, respectively. Laboratory investigations on admission revealed a leukocyte count of $7.70 \times 10^9/\text{l}$, erythrocyte count of $4.33 \times 10^{12}/\text{l}$, platelet count of $313.0 \times 10^9/\text{l}$, serum total protein of 69.0 g/l, serum albumin of 41.0 g/l, serum creatinine of 53.9 $\mu\text{mol/l}$, uric acid of 261.7 $\mu\text{mol/l}$, and serum total cholesterol of 3.9 mmol/l. Urinalysis revealed a protein concentration of 38 mg/ml, with sediment containing 30–49 erythrocytes, and 5–9 leukocytes per high-power field. Urinalysis revealed protein excretion of 0.5 g/day. Her creatinine clearance (24-h) was 125.5 ml/min per 1.73 m². Immunology studies revealed C3 at 86 mg/dl (normal range 65–135), C4 at 11 mg/dl (13–35), CH50 at 30.1 U/ml (30–45), an antinuclear antibody titer <160 $\times$, negativity for anti-DNA antibodies, and the serum titer for myeloperoxidase (MPO)-ANCA was 55EU (STACIA, MEBLUX test MPO-ANCA, normal range <3.5 U/ml), while that for serine proteinase 3 (PR3)-ANCA was <10EU. Prothrombin time and activated partial thromboplastin time were both normal (Fig. 1). On the second hospital day, a renal biopsy was performed (Fig. 2). Light microscopy (LM) revealed 28 glomeruli, three of which showed necrotizing crescents, three showed cellular crescents, two showed fibrocellular crescents, and there was one global sclerotic glomerulus. In other glomeruli, mild proliferation of mesangial cells and an increase in mesangial matrices were found, while fibrosis and inflammatory cell accumulation were observed around the glomeruli with crescents. The results of an immunofluorescence study were negative, and electron-dense deposits were observed by electron microscopy. The patient was diagnosed with ANCA-associated GN based on proteinuria, high serum titers of ANCAs, and pauci-immune necrotizing GN in pathological findings.

The patient was treated with combination therapy, consisting of methylprednisolone (500 mg/day $\times$ 3 days) together with urokinase (UK) pulse at 120,000 U/day i.v. bolus for three consecutive days followed by daily PSL (prednisolone; 40 mg/day), MZB (150 mg/day), warfarin (1 mg/day), and dilazep hydrochloride (200 mg/day). At 2 months after treatment, urinary protein excretion (0.1–0.15 g/day) was decreased and the hematuria had disappeared, while the serum titer of ANCAs was also decreased (Fig. 1). No signs of liver dysfunction, uricacidemia, or leucopenia were observed. The dose of prednisolone was tapered, and proteinuria and hematuria later disappeared at 9 months after treatment. No proteinuria or hematuria was observed during a 1-year follow-up with prednisolone (7.5 mg/day) treatment.

Discussion

ANCAs are characteristic markers of small vessel vasculitis, including granulomatosis with polyangiitis, microscopic polyangiitis, eosinophilic granulomatosis with polyangiitis, and idiopathic pauci-immune necrotizing GN, for which the term ANCA-associated vasculitides has been widely accepted. ANCA-associated GN is also the most common finding in very elderly patients biopsied for acute kidney injury, although it is rare in childhood [1, 7].

As to the clinical features at presentation, Hattori et al. investigated 31 patients with ANCA-associated GN (21 patients with MPA and 10 patients with pauci-immune necrotizing GN) and reported that pulmonary hemorrhage, purpuric rash, arthralgias, arthritis involving both large and small joints, and abdominal pain with or without gastrointestinal bleeding were observed frequently in 21 patients with MPA, whereas none of the 10 patients with pauci-immune necrotizing GN developed the above extrarenal vasculitic diseases during the follow-up period. Of the 10 patients with pauci-immune necrotizing GN, 10 had hematuria, six had nephrotic syndrome, and seven had renal failure that required dialysis. Further, of the 31 patients, 21 presented with rapidly progressive nephritic syndrome and 4 presented with chronic nephritic syndrome. As to prognosis, nine of 31 patients progressed to ESRD; of these nine patients, four required dialysis during the initial phase of therapy and did not recover renal function, three progressed to ESRD with episodes of relapse, and the remaining two progressed to ESRD without active disease or relapse [2]. Other studies have revealed that ANCA-associated GN progresses to renal failure in 20–40 % of patients; thus, it is important to identify patients with ANCA-associated GN and to manage treatment in the early stages of the disease [1, 3–6]. Our case was identified by SUS and was asymptomatic.

Fig. 1 Clinical course of the present patient. *PSL* prednisolone, *MPO-ANCA* myeloperoxidase-antineutrophil cytoplasmic antibody

Fig. 2 **a** Fibrosis and inflammatory cell accumulation can be observed around the glomeruli with crescents. LM: Masson stain ×100. **b** The cellular crescents and segmental necrotizing lesions can be seen in the glomeruli. LM: PAS stain ×400. **c** Segmental sclerosis and fibrocellular crescents can be observed in the glomeruli, and inflammatory cell accumulation is visible around the glomeruli. LM: PAS stain ×200

Although our case had pauci-immune necrotizing GN, the patient did not show nephrotic syndrome, had only mild proteinuria and hematuria, and maintained normal renal

function. Therefore, the ANCA-associated GN in this patient can be said to have been detected in the early stage by SUS.

With regard to SUS in Japan, the Japanese Ministry of Education began its mass urine screening program for school children aimed at the early detection of insidious renal diseases in 1974 [8, 9]. The procedure operates as follows: yearly urinary screening tests are performed for first- to ninth-grade school children. A first morning urine sample is collected at home in a small plastic bottle. First morning urine is used to exclude a positive result in children with orthostatic proteinuria or floating kidney. The children bring the samples to school, which are then transferred to a screening laboratory for testing. Simple dipstick methods are used to detect proteinuria and hematuria. When a urine test is positive, a second test is performed in the same manner. If this is abnormal, urine sediments are checked (a third test) microscopically and diagnostic steps taken to determine the cause of the urinary abnormalities. Iitaka et al. [10] reported that urinary abnormalities were more frequently detected in junior high school children than in primary school children, with the detection rates in the respective groups being 4.44 and 1.25 % at first screening, 0.42 and 0.29 % at second screening, and 0.34 and 0.23 % at third screening. Murakami et al. [8] reviewed the details of children with abnormalities at the third examination. Among these, the prevalence of nephritis was 1 %, urinary tract infection 2 %, and suspected nephritis 3 %. We found that early identification by SUS may enable the early management and subsequent improvement in prognosis of membranoproliferative glomerulonephritis (MPGN) [9]. The present case, with ANCA-associated GN, suggests that SUS is effective in the early identification of ANCA-associated GN in children. We, therefore, believe that urinary screening programs, such as SUS, are necessary to identify patients with early-stage ANCA-associated GN.

It is impossible to measure ANCA titers in all children found to have urinary abnormalities by SUS to detect early-stage ANCA-associated GN. In our case, serum Cr level was slightly increased compared with the normal range. Thus, we think that it may be necessary to examine the ANCA titers for children found to have urinary abnormalities by SUS and increased serum Cr levels.

There are currently no guidelines for the dosage or duration of immunosuppressant treatment in children with ANCA-associated GN, and the recommended regimens are similar to those for adults [11]. Hattori et al. [2] pointed to the beneficial effects of cyclophosphamide (CPM) over corticosteroids alone on remission rate, risk of relapse, and patient survival, but cautioned an association with severe and potentially lethal side effects including oncogenic risk. Valentini et al. [3] showed that CPM and corticosteroids were effective for the acute phase of ANCA-associated GN and found that the use of azathioprine or methotrexate were

effective for maintenance treatment in an effort to provide sustained cytotoxic protection with reduced CPM toxicity.

Our case was treated with multi-drug combination therapy, consisting of methylprednisolone and UK pulse, prednisolone, MZB, warfarin, and dilazep hydrochloride. At 2 months after treatment, urinary protein excretion was decreased and hematuria had disappeared, while the serum titer of ANCA was also decreased. The dose of prednisolone was tapered, and proteinuria and hematuria later disappeared at 9 months after treatment. No signs of liver dysfunction, uricacidemia, or leucopenia were observed. This multi-drug combination therapy, including MZB, was effective and safe for an 11-year-old girl with ANCA-associated GN. UK, a plasminogen activator derived from fresh human urine, first attracted attention as a therapeutic agent for thrombotic diseases such as cardiovascular diseases or cerebral thrombosis. The molecular weight of UK is 54,000, and it has been used in patients with chronic GN, including IgA nephropathy with fibrinogen or fibrin deposits detected by immunofluorescence. The rationale for such treatment was as follows: (1) the defibrinating activity of UK was stronger than of anti-coagulants; (2) specific accumulations of UK were seen in the kidney and liver despite a very short turn over rate; and (3) adverse effects were very rare even if UK was administered for a long period [12, 13]. We have reported that the improvement of hypercoagulable states and acute inflammatory responses, and the suppression of sclerotic changes in glomeruli were found in HSPN patients with crescent GN. We, therefore, believe that UK is effective in the treatment of hypercoagulable states and acute inflammatory responses in ANCA-associated GN with crescent GN.

In addition, MZB is a new immunosuppressive agent developed in Japan that has been reported to selectively inhibit inosine monophosphate dehydrogenase and guanosine monophosphate synthetase, resulting in the inhibition of T-cell and B-cell proliferation [7, 14]. Previous studies have demonstrated its efficacy and safety in the treatment of ANCA-associated renal vasculitis [7]; however, there have been no reports on the application of multi-drug combination therapy including MZB for pediatric ANCA-associated GN. In our case, as the patient had entered puberty, we treated her with multi-drug combination therapy, including MZB instead of CPA, in consideration of side effects including gonad functional disorder and oncogenic risk. The present findings suggest that multi-drug combination therapy including MZB may be effective and safe for a patient with early-stage ANCA-associated GN detected by SUS. However, to evaluate the efficacy of multi-drug combination therapy including MZB for pediatric ANCA-associated GN, it is necessary to observe larger numbers of patients treated with multi-drug combination therapy including MZB with long-term follow-up.

As to the prognosis of ANCA GN, it has been reported that the prognosis of systemic ANCA GN is poor [1–4]. However, Weidner et al. reported that the patients with “renal-limited” ANCA-associated vasculitis had good outcomes compared with patients with MPA or granulomatosis with polyangiitis [15]. Thus, we think that in our case, both the “renal-limited” ANCA-associated vasculitis and the early detection and treatment afforded by SUS were factors indicating a good prognosis.

In conclusion, our results indicate that SUS may be effective in allowing the early identification and treatment of children with ANCA-associated GN.

Conflict of interest We have no conflicting interests affecting the present study.

Informed consent Informed consent was obtained from all patients or their parents.

References

1. Davies DJ, Moran JE, Niall JF, Ryan GB. Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology? *Br Med J*. 1982;285(6342):606.
2. Hattori M, Kurayama H, Koitabashi Y. Japanese society for pediatric nephrology antineutrophil cytoplasmic autoantibody-associated glomerulonephritis in children. *J Am Soc Nephrol*. 2001;12(7):1493–5002.
3. Valentini RP, Smoyer WE, Sedman AB, Kershaw DB, Gregory MJ, Bunchman TE. Outcome of antineutrophil cytoplasmic autoantibodies-positive glomerulonephritis and vasculitis in children: a single-center experience. *J Pediatr*. 1998;132(2):325–8.
4. Yu F, Huang JP, Zou WZ, Zhao MH. The clinical features of anti-neutrophil cytoplasmic antibody-associated systemic vasculitis in Chinese children. *Pediatr Nephrol*. 2006;21(4):497–502.
5. Peco-Antic A, Bonaci-Nikolic B, Basta-Jovanovic G, et al. Childhood microscopic polyangiitis associated with MPO-ANCA. *Pediatr Nephrol*. 2006;21(1):46–53.
6. Vizjak A, Rott T, Koselj-Kajtna M, Rozman B, Kaplan-Pavlovic S, Ferluga D. Histologic and immunohistologic study and clinical presentation of ANCA-associated glomerulonephritis with correlation to ANCA antigen specificity. *Am J Kidney Dis*. 2003;41(3):539–49.
7. Hirayama K, Kobayashi M, Hashimoto Y, et al. Treatment with the purine synthesis inhibitor mizoribine for ANCA-associated renal vasculitis. *Am J Kidney Dis*. 2004;44(1):57–63.
8. Murakami M, Yamamoto H, Ueda Y, Murakami K, Yamauchi K. Urinary screening of elementary and junior high-school children over a 13-year period in Tokyo. *Pediatr Nephrol*. 1991;5(1):50–3.
9. Kawasaki Y, Suzuki H, Suzuki J, Nozawa R, Suzuki H. Efficacy of school urinary screening for membranoproliferative glomerulonephritis type 1. *Arch Dis Child*. 2002;86(1):21–5.
10. Iitaka K, Igarashi S, Sakai T. Hypocomplementaemia and membranoproliferative glomerulonephritis in school urinary screening in Japan. *Pediatr Nephrol*. 1994;8(4):420–2.
11. Bemler JH, Andrassy K. Cyclophosphamide treatment in systemic necrotizing vasculitis and lupus nephritis. How long? How much? *Pediatr Nephrol*. 2004;19(9):949–55.
12. Kawasaki Y, Suzuki J, Nozawa R, Suzuki S, Suzuki H. Efficacy of methylprednisolone and urokinase pulse therapy for severe Henoch-Schönlein nephritis. *Pediatrics*. 2003;111(4):785–9.
13. Kawasaki Y, Suzuki J, Suzuki H. Efficacy of methylprednisolone and urokinase pulse therapy combined with or without cyclophosphamide in severe Henoch-Schönlein nephritis: a clinical and histopathological study. *Nephrol Dial Transplant*. 2004;19(4):858–64.
14. Kawasaki Y, Suzuki J, Sakai N, Etoh S, Murai H, Nozawa R, Suzuki H. Efficacy of prednisolone and mizoribine therapy for diffuse IgA nephropathy. *Am J Nephrol*. 2004;24(1):147–53.
15. Sven W, Steffen G, Syrus H, Andrea W, Harald D. ANCA-associated vasculitis with involvement: an outcome analysis. *Nephrol Dial Transplant*. 2004;19(6):1403–11.