

Membranous glomerulopathy in children given allogeneic hematopoietic stem cell transplantation

Graft-versus-host disease (GVHD) is a common complication of allogeneic haematopoietic stem cell transplantation (HSCT), but membranous glomerulopathy (MG) has rarely been described as a manifestation of chronic GVHD. We report 2 cases of MG in children who were given allogeneic HSCT. The clinical findings were characterized by edema of the lower extremities and nephrotic proteinuria in one case; hypertension, haematuria and edema with non-nephrotic proteinuria in the other one. Renal biopsy was consistent with MG and appropriate immunosuppressive therapy was prescribed. Both patients achieved complete remission and are alive without renal disease 4 and 2 years after the diagnosis of MG, respectively. The normal levels of albumin and non-nephrotic proteinuria in one of the 2 cases raise the question of whether the real incidence of MG after HSCT is underestimated. Therefore, we strongly suggest regular urine analysis during the follow up of children given HSCT to timely diagnose MG.

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Chronic graft-versus-host disease (cGVHD) is a complex, multi-organ syndrome developing more than 100 days after allogeneic haematopoietic stem cell transplantation (HSCT).^{1,2} cGVHD accounts for relevant morbidity and mortality in long-term survivors of allogeneic HSCT; it occurs in about 30 to 50% of recipients of transplants from a human leukocyte antigen (HLA)-matched sibling and in 60 to 70% of patients given HSCT from an unrelated donor.^{1,2} The HSCT-related nephropathies are a rare event and include acute renal failure (ARF), haemolytic uremic syndrome (HUS), nephrotic syndrome (NS) and chronic renal failure (CRF).^{3,4} HSCT-related nephropathies can be divided in two groups according to the time of appearance: early-onset and late-onset (before or 150 days after HSCT, respectively). Drugs, especially cyclosporine A (CsA) and infections are responsible for the early-onset nephropathy and lead mainly to ARF and HUS. Conversely, late-onset nephropathies are mainly represented by GVHD-related NS, CRF and irradiation-related nephropathy.^{3,4} NS has been rarely described as late-onset nephropathy after HSCT and the most common pathologic finding sustaining NS is membranous glomerulonephritis (MG).^{5,6} In this report, we describe two cases of children who developed MG, after HSCT from either an unrelated volunteer or an HLA-compatible sibling, respectively. The disease occurred in two patients in whom GVHD had been diagnosed after discontinuation of CsA and resolved after reintroduction of that drug.

Case Reports

Case 1.

C.L. was admitted to a hospital because of mucocutaneous bleeding in April 1999, at the age of 5 years. He was diagnosed as having *refractory anaemia with excess blasts in transformation* and in September 1999 he underwent HSCT from an HLA-compatible unrelated donor. He was conditioned with busulphan (16 mg/Kg over 4 days), cyclophosphamide (120 mg/Kg over 2 days) and melphalan (140 mg/m² in single dose). CsA, short-term

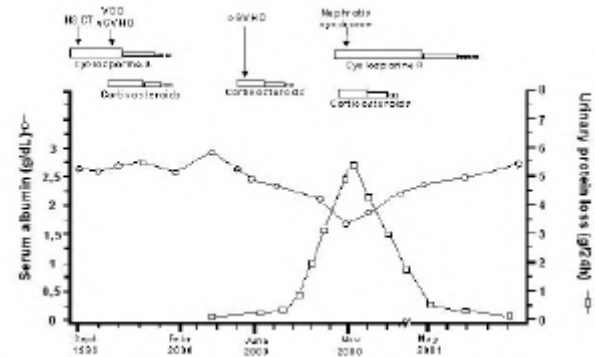


Figure 1. Case 1. Correlation among clinical features, laboratory data and therapies administered after haematopoietic stem cell transplantation. Symptoms related to nephrotic syndrome and renal damage improved after reintroduction of Cyclosporine-A. HSCT: haematopoietic stem cell transplantation. VOD: veno-occlusive disease. aGVHD: acute graft-versus-host disease. cGVHD: chronic graft-versus-host disease.

methotrexate and anti-thymocyte globulin were administered as GVHD prophylaxis. The clinical course after HSCT is described in Figure 1. In the immediate post-transplant period, the child developed liver veno-occlusive disease and grade II acute GVHD, with skin and gut involvement, which resolved after treatment with steroids. Seven months after the allograft, limited chronic GVHD of the skin with lichenoid lesions was diagnosed through a biopsy and it was successfully treated with a 3-month course of steroids. In November 2000, eight months after CsA withdrawal, he presented edema of the lower extremities without hypertension. The laboratory investigations showed: serum albumin 1.7 g/dL, serum total protein 4.2 g/dL, total cholesterol 343 mg/dL and triglycerides 481 mg/dL. Renal and liver function, serum immunoglobulins and complement levels (C3, C4) were normal. Antinuclear antibodies and anti-DNA were not detected. Tests for HBsAg, anti-cytomegalovirus (CMV) IgM and IgG, anti-Epstein Barr virus (EBV) IgM and IgG, anti-HCV and anti-HIV were negative. Urine analysis showed microscopic haematuria and nephrotic proteinuria: the 24-hour urinary albumin loss was 5 g (Figure 1). NS was diagnosed and renal biopsy was performed. On light microscopy, the findings of the specimen were consistent with a diagnosis of MG: mild thickening of the capillary walls, in absence of interstitial infiltrate or tubular atrophy, whereas both matrix and mesangial cells, as well as vessels, were normal. Immunofluorescence showed subepithelial granular deposits of IgG (+++) and C1q (+) along the capillary walls; C3, IgA and IgM were absent (*not shown*). The patient was treated with CsA (5 mg/Kg/day) and prednisone (1 mg/Kg/day), with improvement of the signs and symptoms of disease. Prednisone dosage was gradually tapered after one month of treatment, and CsA after 6 months. Corticosteroid and CsA therapy was withdrawn completely after 3 months and after 9 months, respectively (Figure 1). The patient had complete remission and showed no signs of renal disease. He is alive and well four years later.

Case 2. D.G., an 11-year-old male, was diagnosed as having CD10+ Acute Lymphoblastic Leukaemia (ALL) in January 2001 and was treated according to the ALL 2000 protocol for standard risk patients of the Paediatric

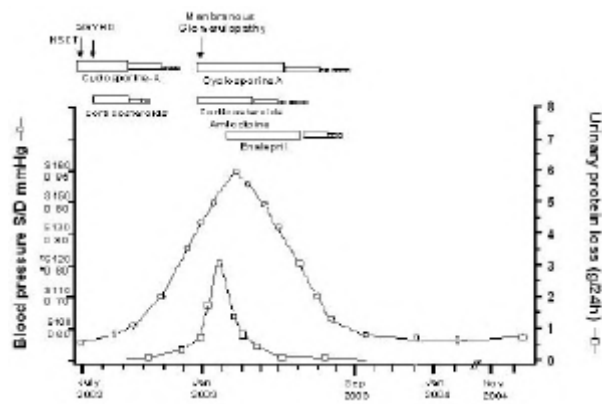


Figure 2. Correlation among clinical features, laboratory data, blood pressure and medications given after haematopoietic stem cell transplantation. Membranous glomerulopathy occurred in absence of hypoalbuminemia and nephrotic proteinuria. Renal damage promptly improved after reintroduction of Cyclosporine-A. *90th Percentile of Systolic (S) and Diastolic (D) blood pressure at patient's age. HSCT: haematopoietic stem cell transplantation. aGVHD: acute graft-versus-host disease.

Haematology-Oncology Italian Association (AIEOP). Fifteen months after diagnosis, he presented isolated central nervous system relapse, and was successfully re-induced into remission with the AIEOP REC-ALL 2003 protocol. In July 2002, he underwent HSCT from an HLA-compatible family donor. He was conditioned with total body irradiation (TBI, 1200 cGy over 6 fractions), thiotepa (10 mg/Kg) and cyclophosphamide (60 mg/Kg/day for 2 consecutive days). CsA and short-term methotrexate were administered as GVHD prophylaxis. The clinical course after HSCT is described in Figure 2. After transplantation, the patient developed grade II acute GVHD, with skin and liver involvement. Treatment with steroids and CsA successfully resolved acute GVHD, which was followed by the appearance of minimal lichenoid skin lesions suggestive for chronic GVHD and not requiring immune-suppressive therapy. In January 2003, one month after CsA withdrawal, he presented slight edema of the eyelids and the lower extremities without hypertension. The laboratory investigations revealed: serum albumin 3.4 g/dL, total protein 5.2 g/dL, total cholesterol 193 mg/dL and tryglicerides 65 mg/dL. Renal and liver function, serum immunoglobulins and complement levels (C3, C4) were normal. Antinuclear antibodies and anti-DNA, tests for HBsAg, anti-CMV IgM, anti-EBV IgM and anti-HCV were negative. CMV antigenemia was negative, as well as neither BK virus nor adenovirus were isolated from urine. Urine analysis showed microscopic haematuria and non-nephrotic proteinuria: the 24-h urinary albumin loss was 1 gram. After 3 weeks, he showed an increase of the urinary albumin loss, about 3 grams per day, with a normal level of serum albumin and creatinine, macroscopic haematuria and hypertension (150/85 mm Hg) (Figure 2). Laboratory data indicated progressive development of moderate anaemia (Hb 8.1 g/dL) without evidence of intravascular haemolysis, as both aptoglobin serum levels were normal and direct antiglobulin test was negative. Because of a moderately low platelet count (PLT $91 \times 10^9/L$) persisting after the allograft, renal biopsy was postponed and the patient was treated with prednisone (0.75 mg/Kg/day), CsA (2 mg/Kg/day) and amlodipine besylate (5 mg/day). In the following weeks edema disappeared, the 24-h urinary albumin loss became 1.3 grams, while microscopic

Figure 3. Light microscopy of case 2. Masson stain 500X. Diffuse thickening of the capillary walls.

haematuria persisted, together with hypertension, requiring the addition of enalapril (10 mg/day). After 6 weeks from the onset of renal symptoms, renal biopsy with laparoscopic technique was performed. On light microscopy, the specimen showed thickening of the capillary walls with small areas of interstitial fibrosis and tubular atrophy. Conversely, both mesangium and matrix were normal and endothelial damage was absent (Figure 3). Immunofluorescence showed subepithelial granular deposits of IgG⁺ C3⁺ and C4⁺ in most glomeruli. No deposits were observed along the capillary walls. These findings were consistent with a diagnosis of GVHD-related MG. Corticosteroid therapy and amlodipine were completely withdrawn after 6 months, and CsA and enalapril after 8 months (Figure 2). At last follow-up, 2 years after diagnosis of MG, the patient is well, without clinical signs or symptoms of renal damage.

Discussion

GVHD-related MG after allogeneic HSCT is a rare event^{3,4} and even more rarely it has been reported in young age. Here, we describe its occurrence in two children 26 and 6 months, respectively, after the allograft. In the period between January 1999 and December 2002, the HSCT Unit of Paediatric Haematology-Oncology of IRCCS Policlinico San Matteo, Pavia, performed 248 allografts in children (120 from an HLA-identical sibling, 17 from an HLA-partially matched relative and 111 from an unrelated volunteer) and 23 cases of cGVHD were diagnosed. We have found only two children with GVHD-related MG, corresponding to 9% of cGVHD cases.

Late-onset, HSCT-related nephropathy occurs 150 days after transplantation and is mainly represented by GVHD-related NS, CRF and TBI-induced nephritis.^{3,4} In case 1, the patient had not been conditioned with TBI and the renal biopsy findings were consistent with MG. Other causes of NS such as infectious diseases or cancer or iatrogenic damage could be excluded. Concerning patient 2, although he was conditioned with TBI, he showed a different pattern of MG from radiation nephropathy, which includes mesangiolytic, tubulointerstitial scarring, glomerular endothelial injury and important fibrosis.

The altered immune-regulation of cGVHD could rea-

sonably have been responsible for an immune-mediated MG in our cases. The mechanism responsible for MG as a manifestation of cGVHD is still unclear. Hiesse *et al.*⁷ and Gomez-Garcia *et al.*⁸ described GVHD-related MG for the first time and suggested an etiologic relationship between HSCT, cGVHD and NS. Successively, other cases of NS following HSCT have been reported.^{5,6,9-17} In most cases, biopsy specimens showed features of MG. Minimal change, segmental glomerular sclerosis and membranoproliferative glomerulonephritis have also been described.^{8,12,15,18} The involvement of circulating immune complexes has been hypothesized in previous reports, but direct GVHD-mediated damage cannot be excluded because in most cases circulating autoantibodies, such as anti-DNA, have not been detected. Moreover, the majority of patients had a history of acute and/or chronic GVHD, this suggesting that GVHD and MG are causally linked.^{5,8,10-17} Experimental data supporting an etiologic relationship between GVHD and the development of MG have also been reported in murine models of GVHD associated with heavy proteinuria.¹⁹ In addition, the possible role of withdrawal of CsA administered as prophylaxis of GVHD should be considered. In fact, after either its re-introduction or increased dosage, clinical improvement was achieved in many patients with MG, as observed also in our patients.^{5,12,15} This latter observation suggests that glomerular lesions are likely induced by donor T cells, their resolution being favoured by the immunosuppressive role of CsA. This drug inhibits interleukin and gamma-interferon production by donor T-lymphocytes. These cytokines may be responsible for the altered glomerular permeability leading to proteinuria as in vitro demonstrated by Seconi *et al.*²⁰ In conclusion, although MG is an unusual manifestation of cGVHD, it should be suspected in all patients with hypoalbuminemia and nephrotic proteinuria developing after CsA withdrawal. In addition, since MG can occur with different clinical patterns, the patients with non-nephrotic proteinuria, i.e., normal levels of albumin, may remain undiagnosed and underestimated. Therefore, we recommend that regular urine analysis be included in the follow up of children HSCT recipients, with the aim of identifying all cases of MG, including those with slight symptoms, so that an appropriate and timely therapy can be administered and immune-suppression be resumed.

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