Nephrotic syndrome: an under-recognised immune-mediated complication of non-myeloablative allogeneic haematopoietic cell transplantation

Nephrotic syndrome (NS) is an extremely rare complication of allogeneic haematopoietic cell transplantation (HCT) that usually occurs in association with chronic graft-versus-host disease (C-GVHD). We observed an unexpectedly high incidence of NS in a cohort of 163 consecutive patients undergoing non-myeloablative HCT from a related human leucocyte antigen-compatible donor. Seven patients developed NS at a median 318 d post-transplant (range 119–1203 d; cumulative incidence 6Æ1%). The median age at onset of NS was 46 years (range 33–59 years); three of the seven patients had no evidence of C-GVHD while four had accompanying limited C-GVHD. At diagnosis, median proteinuria was 16Æ5 g/24 h (range 3–24 g/24 h). Renal biopsy was performed in four cases and revealed membranous nephropathy. NS was not always associated with other symptoms of C-GVHD, and in contrast to previous reports, usually did not improve with the re-initiation of aggressive immunosuppression, resulting in progressive renal failure necessitating dialysis in three of seven cases. Membranous nephropathy resulting in NS is a previously unrecognised and clinically significant complication of non-myeloablative HCT.

Keywords: stem cell transplantation, nephrotic syndrome, membranous glomerulonephritis, non-myeloablative stem cell transplantation.

Received 11 May 2005; accepted for publication 11 July 2005
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Summary

Nephrotic syndrome (NS) is an extremely rare complication of myeloablative allogeneic haematopoietic cell transplantation (HCT) that usually occurs in association with chronic graft-versus-host disease (C-GVHD). We observed an unexpectedly high incidence of NS in a cohort of 163 consecutive patients undergoing non-myeloablative HCT from a related human leucocyte antigen-compatible donor. Seven patients developed NS at a median 318 d post-transplant (range 119–1203 d; cumulative incidence 6Æ1%). The median age at onset of NS was 46 years (range 33–59 years); three of the seven patients had no evidence of C-GVHD while four had accompanying limited C-GVHD. At diagnosis, median proteinuria was 16Æ5 g/24 h (range 3–24 g/24 h). Renal biopsy was performed in four cases and revealed membranous nephropathy. NS was not always associated with other symptoms of C-GVHD, and in contrast to previous reports, usually did not improve with the re-initiation of aggressive immunosuppression, resulting in progressive renal failure necessitating dialysis in three of seven cases. Membranous nephropathy resulting in NS is a previously unrecognised and clinically significant complication of non-myeloablative HCT.

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Summary

Nephrotic syndrome (NS) is an extremely rare complication of myeloablative allogeneic haematopoietic cell transplantation (HCT) that usually occurs in association with chronic graft-versus-host disease (C-GVHD); (Haseyama et al, 1996; Nergizoglu et al, 1999; Oliveira et al, 1999; Imai et al, 2000; Lin et al, 2001; Seconi et al, 2003). Membranous nephropathy, characterised by glomerular basement membrane thickening with minimal to no cellular proliferation or infiltration is the most frequently reported underlying histology (Gomez-Garcia et al, 1988; Hiesse et al, 1988; Muller et al, 1989; Barbara et al, 1992; Sato et al, 1995; Haseyama et al, 1996; Yorioka et al, 1998; Nergizoglu et al, 1999; Ohsawa et al, 2000; Ishizuka et al, 2001; Lin et al, 2001; Rossi et al, 2001; Yu et al, 2001; Bernis et al, 2003; Kim et al, 2003; Lee et al, 2003; Miyazaki et al, 2003; Park et al, 2004; Tsutsumi et al, 2004), although other pathologies, such as minimal change disease, have also been described (Walker et al, 1995; Oliveira et al, 1999; Chien et al, 2000; Ishizuka et al, 2001; Akar et al, 2002; Suehiro et al, 2002; Kimura et al, 2003; Chan et al, 2004; Morisada et al, 2004; Sato et al, 2004). Electron microscopy usually reveals immune complex deposition along the subepithelial aspect of the glomerular basement membrane. The temporal association with C-GVHD and an immune complex mediated pathophysiology suggest that post-transplant NS represents a humoral manifestation of C-GVHD. The majority of reported cases had a relatively benign course with resolution or substantial improvement of NS following the initiation of immunosuppressive therapy. There is only one report of NS occurring after non-myeloablative HCT, in a patient with paroxysmal nocturnal haemoglobinuria (PNH) who received busulphan/fludarabine/anti-thymocyte globulin (ATG)-based conditioning (Lee et al, 2003). Here we report an unexpectedly high incidence of NS in a cohort of 163 consecutive patients undergoing allogeneic HCT from a human leucocyte antigen
(HLA)-compatible related donor following cyclophosphamide/fludarabine-based conditioning. This is the largest case series describing NS after allogeneic HCT and the first detailed report characterising this complication in the non-myeloablative setting.

**Study design**

**Patients and donors**

From March 1997 to May 2003, 163 consecutive patients (median age 48 years, range 14–71 years) underwent transplantation following non-myeloablative conditioning with fludarabine and cyclophosphamide on experimental protocols approved by the National Heart, Lung and Blood Institute (NHLBI) Institutional Review Board. The cohort included patients with haematological malignancies (n = 48), metastatic solid tumours (n = 101, including 65 patients with renal cell carcinoma (RCC), 12 patients with melanoma and 24 patients with other solid tumours), and non-malignant haematological diseases including severe aplastic anaemia (n = 9) and PNH (n = 5). The preparative regimen consisted of cyclophosphamide 60 mg/kg on days −7 and −6 followed by fludarabine 25 mg/m² × 5 d (days −5 to −1). ATG (40 mg/kg/d × 4 doses, days −5 to −2) was added to the conditioning regimen in patients receiving a single HLA-antigen-mismatched graft or those who had a prior history of multiple red blood cell or platelet transfusions (n = 18). On day 0, an unmanipulated granulocyte colony-stimulating factor-mobilised peripheral blood stem cell allograft was infused from an HLA-identical (n = 157) or single antigen-mismatched (n = 6) related donor (sibling in all but three patients). Ciclosporin (CSA), given alone (n = 66) or combined with mycophenolate (MMF; n = 82) or mini-dose methotrexate (5 mg/m² 24 h/days 1, 3, 6; n = 15) was used as GVHD prophylaxis. In all three groups, decisions regarding discontinuation of immunosuppression were based on the degree of donor T-cell chimaerism, presence of GVHD, and disease status (in those with malignant diseases). In the absence of grade II–IV acute GVHD and disease progression, CSA (±MMF) was tapered slowly beginning on day +60 and discontinued by day +100. Patients with disease progression or mixed T-cell chimaerism at or beyond day +30 had their immunosuppression tapered more rapidly. Patients with disease progression or those with persistent mixed T-cell chimaerism after immunosuppression withdrawal were eligible to receive monthly escalating doses of donor lymphocyte infusions.

**Evaluation of nephrotic syndrome**

Unexplained hypoalbuminaemia or proteinuria led to a work-up for NS. A diagnosis of NS was confirmed by urinary protein excretion of 3 g/24 h in the presence of hypoalbuminaemia (serum albumin <30 g/l) and peripheral oedema.

**Results**

Seven of 163 consecutive patients (median age 46 years, range 33–59 years) undergoing non-myeloablative HCT developed nephrotic range proteinuria at a median 318 d (range 119–1203) (Table I) post-transplant (cumulative incidence 6.1%, with a median follow up of 2.8 years). One patient underwent transplantation for secondary acute myeloid leukaemia, one for chronic phase chronic myeloid leukaemia, one for adrenal cortical carcinoma, and four for metastatic RCC. At the time NS was diagnosed, three patients had no overt manifestations of C-GVHD, while four had limited C-GVHD (manifested by oral mucosal or limited skin involvement). At diagnosis, median proteinuria was 16.5 g/24 h (range 3–24 g/24 h) and peak proteinuria ranged from 3 to 30 g/24 h (median 17 g/24 h); all seven patients had normal pretransplant renal function (median serum creatinine 88.4 μmol/l, range 44.2–106.1 μmol/l) with no evidence of proteinuria (Table I). Renal biopsy was performed in four of seven cases (including an autopsy examination in one case); systemic anticoagulation to treat pulmonary embolism precluded renal biopsy in two patients, while a biopsy was deferred in one patient (patient 4) who had rapid improvement of proteinuria and resolution of NS shortly after diagnosis. Histopathological evidence of membranous nephropathy was observed in all four biopsy specimens, with immune complex deposition observed along the glomerular basement membrane on electron microscopy. Common causes of secondary membranous nephropathy including viral infections, drugs and collagen vascular diseases were excluded in all cases. Despite a similar incidence of C-GVHD (data not shown), no cases of NS occurred in a cohort of 118 patients undergoing myeloablative HCT (1280 cGy TBI + 120 mg/kg cyclophosphamide) at the NHLBI during the same time period.

Nephrotic syndrome was associated with significant morbidity. Four patients (patients 1, 2, 3 and 6) developed thromboembolic complications following a diagnosis of NS, with two patients each developing pulmonary embolism and catheter-related thrombosis. Three patients (patients 1, 2 and 5) developed progressive renal failure with persistent proteinuria and ultimately required dialysis at 412, 376 and 274 d following the diagnosis of NS. Although all patients were treated with systemic immunosuppressive therapy, only two patients (patients 4 and 6) had complete resolution of proteinuria with preservation of renal function following treatment with corticosteroids while one patient (patient 3) had a transient decrease (<1 month) in proteinuria (from a peak of 17 g/24 h to 4.5 g/24 h) following treatment with corticosteroids, CSA, rituximab, intravenous immunoglobulin and plasma exchange. In the remaining four patients, proteinuria did not respond to multiple interventions including treatment with corticosteroids (all four patients), CSA (all four patients), or rituximab (one patient). To date, five of the seven patients who developed NS have died (two from progression of their underlying malignancy, one from central nervous system complications related
Table I. Baseline characteristics and outcome in patients who developed nephrotic syndrome (NS).

<table>
<thead>
<tr>
<th>PN</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Pretransplant serum creatinine ((\mu)mol/l)</th>
<th>Pretransplant proteinuria</th>
<th>Onset NS (days post-transplant)</th>
<th>C-GVHD</th>
<th>Active C-GVHD at NS onset</th>
<th>Peak proteinuria (g/24 h)</th>
<th>Pretransplant CCR (ml/s)</th>
<th>CCR at NS diagnosis (ml/s)</th>
<th>Progressive renal failure</th>
<th>Time to dialysis (days from diagnosis of NS)</th>
<th>Treatment</th>
<th>Renal biopsy</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55</td>
<td>M</td>
<td>RCC</td>
<td>80</td>
<td>No</td>
<td>318</td>
<td>Limited onset day 161</td>
<td>Yes</td>
<td>30</td>
<td>1.5</td>
<td>1</td>
<td>Yes</td>
<td>412</td>
<td>C, PR, MG</td>
<td>Died, dialysis complications, day 1496</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>F</td>
<td>RCC</td>
<td>88</td>
<td>No</td>
<td>326</td>
<td>Limited onset day 150</td>
<td>Yes</td>
<td>22</td>
<td>1.5</td>
<td>1.1</td>
<td>Yes</td>
<td>376</td>
<td>C, PR, R, MG</td>
<td>Died, dialysis complications, day 718</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>55</td>
<td>M</td>
<td>RCC</td>
<td>106</td>
<td>No</td>
<td>816</td>
<td>Limited onset day 790</td>
<td>Yes</td>
<td>20</td>
<td>1.0</td>
<td>0.5</td>
<td>No</td>
<td>–</td>
<td>C, R, PR, MG, IVIG</td>
<td>Died, disease progression, day 275</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>59</td>
<td>M</td>
<td>RCC</td>
<td>106</td>
<td>No</td>
<td>119</td>
<td>None</td>
<td>None</td>
<td>17</td>
<td>1.3</td>
<td>1.1</td>
<td>No</td>
<td>–</td>
<td>PR, –</td>
<td>Died, disease progression, day 414</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>46</td>
<td>F</td>
<td>Adrenocortical carcinoma</td>
<td>80</td>
<td>No</td>
<td>136</td>
<td>None</td>
<td>None</td>
<td>14</td>
<td>1.4</td>
<td>1.2</td>
<td>Yes</td>
<td>274</td>
<td>C, PR</td>
<td>Died, intracranial haemorrhage/ TTP, day 1245</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>33</td>
<td>M</td>
<td>CML</td>
<td>88</td>
<td>No</td>
<td>186</td>
<td>None</td>
<td>None</td>
<td>11</td>
<td>2.0</td>
<td>1.0</td>
<td>No</td>
<td>–</td>
<td>PR, –</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>43</td>
<td>F</td>
<td>AML</td>
<td>44</td>
<td>No</td>
<td>120*</td>
<td>Limited onset day 103</td>
<td>Yes</td>
<td>3.0</td>
<td>1.9</td>
<td>1.0</td>
<td>No</td>
<td>–</td>
<td>C, PR, MG</td>
<td>Died, intracranial haemorrhage/ TTP, day 1245</td>
<td></td>
</tr>
</tbody>
</table>

*3+ proteinuria associated with hypoalbuminaemia first detected on day 957; 24-h urine collection confirmed NS obtained on day 1203.

RCC, renal cell carcinoma; CML, chronic myeloid leukaemia; AML, acute myeloid leukaemia; MG, membranous glomerulonephritis; C, ciclosporin; R, rituximab; P, plasma exchange; IVIG, intravenous immunoglobulin; PR, prednisone; CCR, calculated creatinine clearance; TTP, thrombotic thrombocytopenic purpura.
to thrombotic thrombocytopenic purpura, and two from complications related to chronic dialysis).

**Discussion**

In contrast to myeloablative HCT where NS is a rare occurrence, we observed a surprisingly high incidence of NS in patients undergoing cyclophosphamide/fludarabine-based non-myeloablative HCT. The relatively aggressive withdrawal of post-transplant immunosuppression to enhance graft-versus-tumour effects may have predisposed to the development of membranous glomerulonephritis following this non-myeloablative transplant approach. It is also possible that host B cells and plasma cells that survive non-myeloablative conditioning play a role in the pathophysiology of this condition. Immune complex deposition, as was observed in the four biopsy specimens obtained from NS patients in this analysis, is a characteristic feature of membranous glomerulonephritis, and dysregulation of antibody-producing cells could lead to this immune-mediated complication. The importance of humoral mechanisms in mediating this type of nephropathy is best illustrated by a parent into F1 (DBA2_C57B6/DBA2 F1) murine transplant model, where persistent host B-cell activation appeared to be necessary for the development of C-GVHD manifestations, including a lupus-like syndrome with membranous nephropathy. In contrast, when the other parent (C57B6) was used as the donor, host B cells were rapidly eliminated and C-GVHD and membranous nephropathy did not develop (Rus et al., 1995). The lack of recipient conditioning in this murine model likens it to non-myeloablative HCT in humans, where antibody-producing host B cells and plasma cells may persist for months, even after other host haematopoietic populations have converted from mixed to full donor chimaerism (Griffith et al., 2005). Delayed donor erythropoiesis and pure red blood cell aplasia are examples of complications mediated by host humoral immunity that may occur with higher frequency after non-myeloablative compared with myeloablative transplantation. In the setting of major-ABO mismatched non-myeloablative transplantation, prolonged anti-donor RBC isohaemagglutinin production by host plasma/and or B cells can lead to delays in donor erythropoiesis and sometimes pure red blood cell aplasia. Although not evaluated in this cohort of patients, attempts to elucidate a possible role for host B cells and plasma cells in the development of NS are in progress.

Although five of seven patients in this cohort had a metastatic malignancy as an underlying diagnosis, the incidence of NS as a paraneoplastic complication of solid tumours is exceedingly low (Keur et al., 1989; Norris, 1993; Ronco, 1999). Furthermore, our series did not include patients with lung and colon cancer, the tumour subtypes most commonly associated with NS. With the exception of patients treated with bevacizumab (a monoclonal antibody against vascular endothelial growth factor), paraneoplastic or treatment-related NS in RCC is extremely rare. None of the patients in our series received bevacizumab; moreover, the incidence of NS in our RCC patients (4/65) was not significantly different from that in patients with other solid tumours (1/36), haematological malignancies (2/48), or non-malignant haematological diseases (0/14) ($P = 0.9$, Fisher’s exact test). Hence, the observation that four of seven cases of NS occurred in RCC patients probably reflects the fact that this was the most common underlying diagnosis ($n = 65$) in patients undergoing non-myeloablative HCT at our centre. However, we cannot completely exclude the possibility that NS in these patients was related to an underlying malignancy.

In contrast to recipients of myeloablative allogeneic HCT reported in the literature, there was no definite correlation between the development of NS and other manifestations of C-GVHD. Furthermore, many of our NS patients suffered significant morbidity from this complication, with persistent proteinuria and a progressive decline in renal function despite the initiation of aggressive immunosuppressive therapy. The anti-CD20 antibody, rituximab, has been shown to be effective in reducing proteinuria in patients with idiopathic NS (Remuzzi et al., 2002) and decreased proteinuria in a patient with post-transplant NS (Ratanatharathorn et al., 2003). In contrast, rituximab therapy was relatively ineffective for both patients who received this agent in our series, including one patient who eventually progressed to end-stage renal failure, requiring dialysis.

In conclusion, membranous nephropathy resulting in NS is a previously unrecognised complication of non-myeloablative HCT associated with significant morbidity, poor response to immunosuppressive therapy and a propensity for progressive renal failure. Host B cells and plasma cells that survive reduced-intensity conditioning and the aggressive withdrawal of post-transplant immunosuppression incorporated into many NST regimens potentially predispose to this complication.

**References**


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