Pediatr Nephrol (2014) 29:2055–2059
DOI 10.1007/s00467-014-2839-y

BRIEF REPORT

Eculizumab in dense-deposit disease after renal transplantation

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Received: 5 June 2013 / Revised: 2 April 2014 / Accepted: 24 April 2014 / Published online: 8 June 2014

Abstract
Background Dense-deposit disease (DDD) is a rare glomerulopathy characterized by electron-dense deposits in the glomerular basement membrane. About 50 % of patients with DDD progress to end-stage kidney disease and require dialysis within 10 years of diagnosis, and the disease often recurs after renal transplantation.
Case-Diagnosis/Treatment We describe a 14-year-old girl with recurrent DDD in her transplanted kidney. Clinical onset was at 8 years of age, when steroid-resistant nephrotic syndrome was diagnosed with microhematuria, severe hypocomplementemia and normal kidney function. Although remission was initially observed after several plasma exchanges, nephrotic proteinuria returned and kidney function further declined 1 year later. The patient received a living-related kidney transplant. Initial allograft function was good, but proteinuria reappeared 3 months after transplantation, accompanied by a slight deterioration in kidney function. After histological confirmation of DDD recurrence and subsequent management with plasmapheresis, the patient was treated for 30 months with eculizumab, a humanized monoclonal antibody that binds to C5 complement protein. This intervention proved effective and resulted in complement inhibition, sustained remission of proteinuria and preservation of renal function. A graft biopsy 6 months later showed no progression of the renal lesions.
Conclusions Early clinical and histological recurrence of DDD in the transplanted kidney in this 14-year-old patient was treated for 30 months with eculizumab. The patient remains asymptomatic, has no proteinuria and her kidney function is intact.

Keywords Dense-deposit disease · Eculizumab · Pediatric renal transplant

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Introduction

Dense-deposit disease (DDD), also known as type II membranoproliferative glomerulonephritis (MPGN II), is an uncommon glomerulopathy affecting mainly young people. It is characterized by complement and electron-dense deposits at the glomerular basement membrane [1] and is considered to be a C3 glomerulopathy. Immunoglobulin-negative and C3-positive MPGN I and MPGN III together with DDD were recently grouped under the term C3 glomerulopathies (C3GN), which emphasizes the pathogenic importance of dysregulation of the alternative complement pathway [2]. Patients with DDD have reduced serum C3 complement, suggesting activation of the alternative pathway of the complement cascade. Furthermore, more than 70 % of patients with DDD have C3 nephritic factor (C3NeF), an autoantibody that binds C3 convertase, stabilizing and protecting it from

enzyme degradation. The presence of C3NeF induces persistent C3 activation, which leads to severe hypocomplementemia [3]. However, the pathophysiological role of C3NeF in the glomerular damage observed in patients with DDD remains unclear. Complement dysregulation in C3GN is also occasionally due to complement gene mutations [4]. More than half of DDD patients progress to end-stage renal disease within 10 years of diagnosis [2]. DDD may progress to end-stage kidney disease (ESKD) more frequently in young females than in other population groups [5], and in patients who undergo transplant biopsy, the average rate of recurrence exceeds 70 %. Disease recurrence leads to a 5-year allograft failure rate of 50 % [2]. There is currently no proven effective therapy for DDD [6].

Eculizumab is a humanized monoclonal antibody that binds to the C5 complement protein and inhibits its cleavage into proinflammatory and prothrombotic C5a and C5b. This binding prevents the generation of the prothrombotic and cytolytic terminal complement complex (C5b-9, membrane attack complex). Successful blockade of C5 in C3GN should prevent C5a formation, resulting in less neutrophil and leukocyte infiltration [6]. The uncontrolled activity of the alternative complement pathway suspected in DDD, along with renal damage in animal models [7] with C5 dysregulation, have stimulated the use of eculizumab in DDD [8].

Case report

We describe a 14-year-old adolescent girl with recurrent DDD in the transplanted kidney. She was 8 years old at the onset of the disease, when nephrotic syndrome, microhematuria and preserved renal function were diagnosed. Severe hypocomplementemia at the expense of the C3 fraction (initial C3 36 mg/dL, range 90–180 mg/dL) was detected at the beginning of the process, together with steroid resistance. Renal biopsy confirmed DDD (Fig. 1a, d, g). She was positive for C3Nef, had normal levels of factor H (CFH), membrane cofactor protein CD46 (MCP), CFI and complement factor B (CFB) and anti-factor H antibody tests were negative. No mutations were detected in the *CFH*, *MCP*, *CFI*, *C3* or *CFB* genes. In addition, no genetic rearrangements in the *CFHR1-5* genes or anomalies in the expression of factor H-related (FHR) proteins in plasma were observed.

The patient was treated with 31 sessions of plasma exchange (PE) for 6 months from December 2007 to June 2008 as follows: three sessions per week for the first 2 weeks (6 sessions), two sessions per week for the following 4 weeks (8 sessions) and one session per week for 17 weeks (17 sessions). This initially led to normalization of proteinuria for 1 year, but nephrotic proteinuria subsequently reappeared (Fig. 2a). Treatment was started with rituximab, which resulted in a partial, transient reduction in proteinuria, and with combined antiproteinuric therapy of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers. One year later (2.5 years after disease onset) her renal function deteriorated rapidly to ESKD with severe proteinuria, and she developed a hypertensive emergency with seizures and severe headaches. She required hemodialysis three times per week for 5 months. After the potential risks of living-related kidney transplantation (due to the high rate of posttransplant recurrence of DDD) were explained to the family, the patient received a kidney transplant from her father, who had no identified complement anomalies (normal plasma C3 values), no hematuria and no proteinuria.

The patient then received immunosuppression therapy for transplantation with basiliximab, steroids, tacrolimus and mycophenolate mofetil, which initially resulted in an excellent allograft function and disappearance of the proteinuria. However, mild proteinuria (0.877 g/24 h, 1,194 mg/24 h/1.73 m^2) was detected 3 months after transplantation. Her urine protein/creatinine ratio was 1 (range <0.2) mg/mg and the microalbumin/creatinine ratio was 0.569 (range <0.030) mg/mg (Fig. 2b) with a slight deterioration in renal function. Her serum creatinine increased from 0.8 to 1.1 mg/dL (71–97 μmol/L) and estimated glomerular filtration rate (eGFR) calculated by the Schwartz formula decreased from 97 to 71 mL/min/1.73 m^2 (1.62–1.18 mL/s/1.73 m^2). A graft biopsy showed the same changes as in the native kidney [mesangial proliferation, capillary thickening (Fig. 1b) and C3 deposits (Fig. 1e), electron-dense deposits in the glomerular basement membrane (Fig. 1h)] confirming the diagnosis of recurrent DDD in the transplanted kidney. There were no histological signs of acute rejection, with negative results for C4d in the peritubular capillaries and IgG, IgM, IgA and fibrinogen tests. The patient was treated once again with PE for a total of 22 sessions in 3 months (3 sessions per week for the first 2 weeks, 2 sessions per week for the following 4 weeks and 1 session per week for 8 weeks), which led to remission of the proteinuria. In view of her reduced quality of life with prolonged PE, we started treatment with eculizumab in an attempt to block uncontrolled complement activation and curtail the kidney damage. Before eculizumab treatment was started, the patient was vaccinated against encapsulated bacteria such as *Neisseria meningitidis* (4 ACWY serotypes) and *Streptococcus pneumoniae* (10 serotypes). A 900-mg dose of eculizumab was administered once a week for 4 weeks, followed by 1,200 mg every 2 weeks for 1 year, every 3 weeks for 1 year and every 4 weeks currently, with good tolerance and no adverse episodes related to this medication. The patient reported acute back pain after the second dose, which resolved with metamizole. Following the initiation of eculizumab therapy, the patient showed complete inhibition of terminal complement (C5b-9) activity, measured as the degree of hemolysis, no proteinuria and a decline in serum creatinine from 1.1 to 0.8 mg/dL (97–71 μmol/L). This improvement in kidney

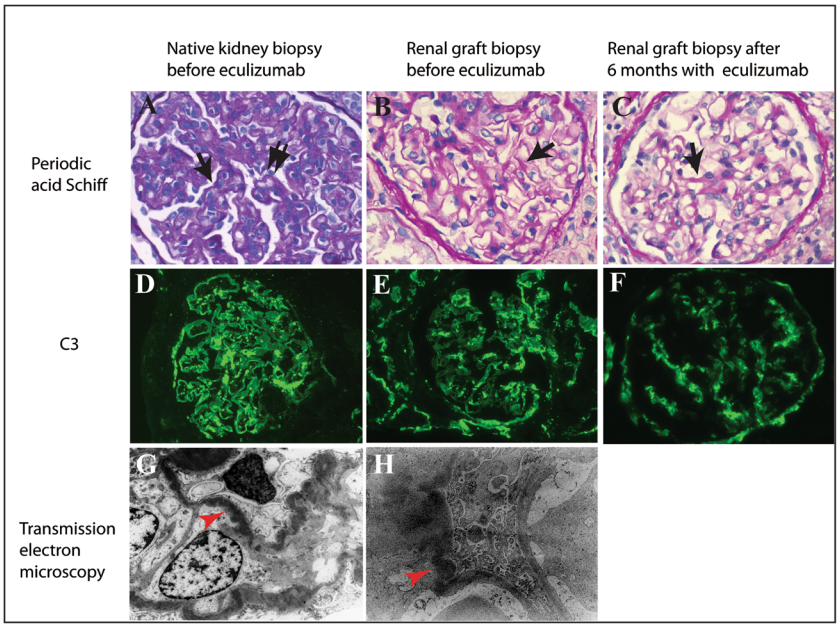

Fig. 1 Light microscopy (LM), immunofluorescence (IF) and electron microscopy (EM) findings in three biopsies.**a**, **d**, **g** Native kidney biopsy: **a** LM, showing dense deposit disease (DDD) with mesangial proliferation and thickened capillary walls (*single black arrow*) with double contour formation (*double black arrow*) [periodic acid–Schiff staining (PAS); magnification 40×], **d** IF, showing bright C3 in the mesangium and along the glomerular capillary walls (20×), **g** EM, showing highly electron-dense intramembranous deposits that coalesce in the glomerular basement (*red arrowhead*) (3,900×). **b**, **e**, **h** Renal graft biopsy before eculizumab: **b** LM, showing recurrent DDD lesions in the transplanted kidney with mesangial proliferation and thickened capillaries (*black arrows*) (PAS; 40×), **e** IF, showing staining for C3, highlighting the glomerular capillary walls [immunoglobulin A (IgA)-, IgG-, IgM- and fibrinogen-negative; C4d-negative] (40×), **h** EM, showing electron-dense intramembranous deposits in the glomerular basement membranes (*red arrowhead*) (6,600×). **c**, **f** Renal graft biopsy after treatment with eculizumab for 6 months: **c** LM, showing thickened capillary walls and mild mesangial hyperplasia (PAS; 40×) (*single black arrow*), **f** IF, showing weaker C3 deposits along capillaries (40×); no material available for EM studies

function persisted throughout the duration of the therapy. The plasma concentration of eculizumab was 220–425 mg/L.

At the time of our last observation after 2 years and 6 months of eculizumab treatment, the patient had a urinary protein/creatinine ratio of 0.1 mg/mg (range 0–0.2) (proteinuria 112 mg protein per 24 h), a microalbumin/creatinine ratio of 0.005 (range <0.030) mg/mg, no microhematuria, persistently low C3 levels (26 and 28 mg/dL, range 90–180 mg/dL) and normal renal functioning with a plasma creatinine level of 0.8 mg/dL and an eGFR of 109 mL/min/1.73 m^2 (1.82 mL/s/1.73 m^2) (Fig. 2). She remains on treatment with tacrolimus, mycophenolate mofetil, prednisone and losartan. A protocol biopsy after 6 months of treatment showed no progression of mesangial proliferation, capillary

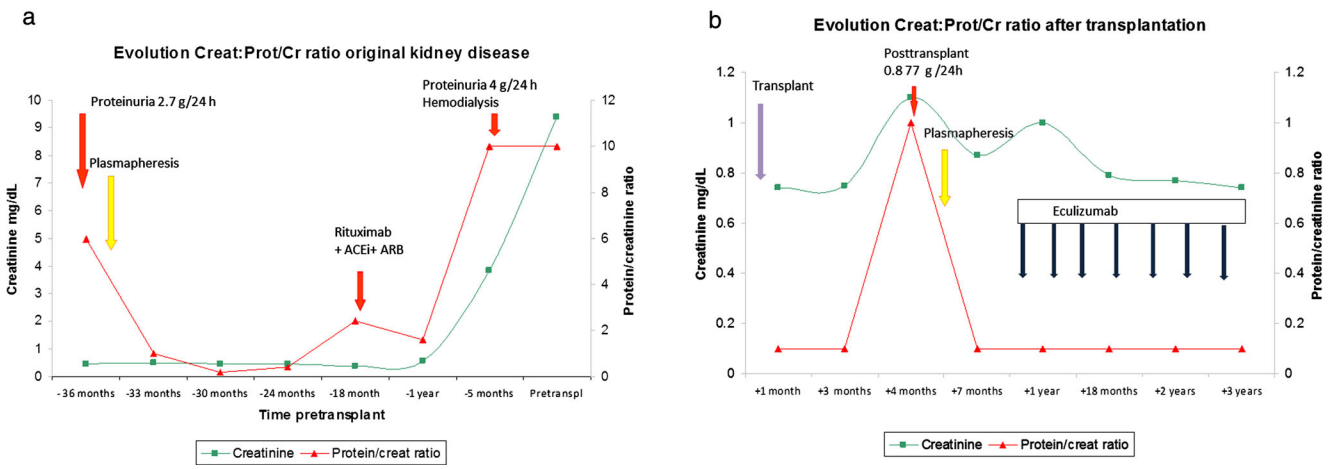

Fig. 2 **a** Changes in serum creatinine (*Creat*) and urine protein/creatinine (*Prot/Cr*) ratio during the 3 years before kidney transplantation, **b** changes in serum Creat and Prot/Cr ratio during the 3 years after transplantation. *ACEi* Angiotensin-converting enzyme inhibitor, *ARB* angiotensin receptor blockers

thickening (Fig. 1c) or C3 deposits (Fig. 1F) compared to the previous biopsy.

Discussion

Dense-deposit disease is a rare glomerulonephritis caused by uncontrolled stimulation of the alternative complement pathway, and 50 % of patients progress to ESKD within 10 years of diagnosis. Allograft survival after renal transplantation is significantly reduced by the high rate of disease recurrence. No therapeutic interventions have consistently improved outcomes for patients with primary or recurrent disease [8].

We present the case of a 14-year-old adolescent girl with early recurrence of DDD after a living donor kidney transplant. At the onset of her disease prior to transplantation, she had responded well to PE, which led us to once again to adopt this therapeutic approach after the recurrence. However, following the subsequent deterioration in renal function leading to ESKD soon after PE was discontinued, we decided that stopping PE was not a safe approach for this patient. However, we firmly believe that indefinite PE has an unacceptably negative impact on the quality of life in young patients, which interferes with normal educational and social development. Disease recurrence leads to a 5-year allograft failure rate of 50 %, and there is currently no proven effective therapy for DDD [6, 8]. In our patient, treatment with eculizumab effectively inhibited the terminal complement cascade, was well tolerated and offered an acceptable quality of life, corroborating the findings reported by other authors. Bomback and colleagues described an open-label, non-blinded proof-of-concept study of eculizumab treatment for DDD and C3 glomerulonephritis, with variable responses. Two of the three patients with DDD who were treated had a favorable clinical course, with kidney function improving in one patient and proteinuria markedly reduced in the other patient who had posttransplant DDD [9]. McCaughan et al. reported a case of aggressive, recurrent DDD after renal transplantation. There was a marked clinical and biochemical response after the administration of eculizumab, but the absence of follow-up biopsies to assess histological improvement is a limitation in this case report [8]. In this same context, Vivarelli and colleagues [10] reported a reduction in mesangial proliferation, capillary thickening and C3 and intramembranous electron-dense deposits after 18 months of eculizumab treatment for DDD in the native kidney. Daina and colleagues [11] reported a good response to eculizumab in a patient with DDD and elevated soluble C5b-9. In their patient, serum creatinine and proteinuria decreased and C5b-9 levels normalized. These authors suggest that elevated serum C5b-9 prior to therapy may be a good predictor of response to eculizumab treatment. The absence of C5b-9 values is a limitation in our case report. However, measuring C5b-9 is complex and nonreproducible, and the results cannot be extrapolated to other patients, as demonstrated in two published studies that have reported widely different values [9, 11]. In our patient the pharmacodynamics test we used showed that terminal complement activity was completely blocked after the first dose of eculizumab. This activity was quantified with a pharmacodynamic assay that quantified complement activity in the patient's serum by measuring the degree of hemolysis.

Both the sustained decrease in serum C3 during our patient's clinical course and C3NeF positivity support the role of complement dysregulation [12] (by fluid-phase dysregulation of the alternative complement pathway) in the pathogenesis of DDD. These findings suggest that controlling excessive complement activation with eculizumab is a potentially effective strategy for managing DDD after transplantation [8, 13].

In light of current knowledge, it is not possible to determine the appropriate duration of treatment. In the patient described by Vivarelli et al. [10], proteinuria increased rapidly when treatment was interrupted after 18 months; when eculizumab therapy was resumed 6 months later, proteinuria decreased. Herlitz et al. [6] reported that after 1 year of eculizumab therapy in three patients with DDD and two with C3GN, renal biopsy findings showed a reduction in active glomerular proliferation and neutrophil infiltration in three of five cases. Recently, Gurkan and colleagues reported a patient with an initial diagnosis of DDD and recurrence of C3GN in the transplant. Partial histopathological and clinical response was observed after 1 year of treatment with eculizumab. The authors suggest that eculizumab blocked the membrane attack complex, but that dysregulation may have persisted in the alternative complement pathway, which prevented the drug from effectively controlling the disease in their patient [14]. The diseases known as C3GN are less homogeneous than the nomenclature suggests, and their response to eculizumab will depend on the intensity and degree of alternative pathway dysfunction. In our patient, we considered that the aggressive nature of her disease in the native kidney, lack of response to corticosteroids or rituximab and the rapid recurrence as reasons to try eculizumab therapy, after all appropriate complement studies had been done as recommended in the recent C3GN consensus report [15].

In summary, treatment with eculizumab was a safe and effective therapeutic option in our adolescent patient with posttransplant recurrence of DDD. After 30 months of eculizumab treatment the patient remains free of proteinuria, her kidney function is normal and progression of the histological lesions after 6 months of her disease has ceased. We observed no undesirable effects or infections associated with the use of eculizumab, and our patient enjoys an excellent quality of life at the time of writing this article.

Acknowledgments We thank M. José Viera and Flora López for their help with nursing care, and K. Shashok for improving the use of English

in the manuscript (with support from the FISEVI Foundation). Medical editing was provided by Bioscript Medical Ltd, supported by Alexion Pharmaceuticals, Inc.

Conflict of interest None.

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