Living kidney transplantation in adult patients with atypical haemolytic uraemic syndrome

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ABSTRACT

Background: Dysregulation of complement activation is the most common cause of the atypical haemolytic uraemic syndrome (aHUS). Many patients with aHUS develop end-stage renal disease and consider kidney transplantation. However, the recurrence rate after transplantation ranges from 45-90% in patients with known abnormalities in circulating complement proteins. It was recently proposed that patients with aHUS should be treated prophylactically with plasma exchange or eculizumab to prevent recurrence after transplantation.

Methods: A case series describing the successful outcome of kidney transplantation without prophylactic therapy in four adult patients with aHUS and a high risk of disease recurrence. Patients received a living donor kidney and immunosuppression consisting of basiliximab induction, low-dose tacrolimus, prednisone and mycophenolate mofetil. Patients received a statin, and were targeted to a low blood pressure preferably using blockers of the renin-angiotensin system.

Results: After a follow-up of 16-21 months, none of the patients developed recurrent aHUS. Also, no rejection was observed.

Conclusions: Kidney transplantation in adult patients with aHUS can be successful without prophylactic eculizumab, using a protocol that minimises cold ischaemia time, reduces the risk of rejection and provides endothelial protection. Our data suggest that in patients with aHUS, controlled trials are needed to demonstrate the optimal strategy.

KEYWORDS

Atypical haemolytic uraemic syndrome, eculizumab, endothelial activation, kidney transplantation

INTRODUCTION

Haemolysis with fragmented erythrocytes, thrombocytopenia and acute renal failure are the hallmark of the haemolytic uraemic syndrome (HUS), and evidence of severe microvascular damage. Diarrhoea-associated HUS occurs mainly in children, and is caused by Shiga-like toxin producing Escherichia coli. In adult patients there is seldom an association with E. coli infection, and the term atypical HUS (aHUS) was coined. Recent studies have documented a mutation in proteins involved in complement C3 and complement regulatory proteins (factor H (CFH), factor I (CFI), factor B (CFB), and membrane cofactor protein (MCP)) in more than 50% of the patients. These genetic abnormalities result in unopposed and excessive activation of the complement system leading to endothelial damage. Atypical HUS is a serious disease, with a mortality rate of 25%. The kidneys are most frequently involved, and kidney injury is usually progressive with more than 50% of patients with aHUS developing end-stage renal disease (ESRD). Kidney transplantation is considered the best treatment modality for patients with ESRD. However, patients with aHUS who undergo kidney transplantation have a poor prognosis. They are at high risk for recurrence of aHUS, which is uniformly associated with graft loss. Especially patients with a documented mutation in complement regulatory proteins (with the exception of MCP) have a risk of recurrence after kidney transplantation that ranges from 45-90%. This process of recurrent aHUS was difficult to treat. Although plasmapheresis and plasma infusion have been used successfully in some patients with aHUS, many patients did not respond (resistant) or needed continued weekly treatment (plasma-dependent). Prophylactic plasma therapy in kidney transplantation seemed to reduce the risk of aHUS recurrence, however without reaching statistical significance in a retrospective...
study. Based on these data, patients with aHUS are often not offered kidney transplantation and remain on dialysis therapy for the rest of their life. The introduction of eculizumab in clinical practice has offered hope for patients with aHUS, particularly for patients who are awaiting a kidney transplantation. Many case reports have documented response to eculizumab in patients with recurrent aHUS after transplantation. Also, many patients have been transplanted successfully with eculizumab given prophylactically. This experience has dramatically changed the prognosis of patients with aHUS. Experts now recommend prophylactic treatment with eculizumab for all patients with aHUS who are at medium or high risk for disease recurrence after transplantation. Also, treatment should be continued for at least 12 months (in medium-risk patients) or even lifelong (in high-risk patients).

The use of eculizumab is associated with enormous costs and limiting the use of eculizumab thus becomes utterly relevant. Moreover, in the Netherlands there is still uncertainty as to whether treatment with eculizumab is reimbursed. However, it is unclear if kidney transplantation without eculizumab is a reasonable approach.

Although mutations in complement regulatory proteins are important in the pathogenesis of aHUS, other factors must also contribute since many persons carrying a disease-causing mutation never develop aHUS. Other factors include polymorphisms in complement regulatory proteins or exogenous factors, particularly factors that either induce complement activation or cause endothelial cell injury. Such factors are always present in transplanted patients and include donor graft injury (related to the donor procurement, cold ischaemia time, and reperfusion injury), acute rejection, use of calcineurin inhibitors (CNI), and hypertension- and lipid-mediated vascular injury. Although not tested in clinical trials, protocols that reduce endothelial injury at the time of transplantation and use rescue eculizumab therapy in case of active, recurrent disease could be an acceptable solution for renal transplantation in patients with aHUS. We preferred transplantation with kidneys from a living donor, because it limits endothelial damage. We are aware that living donor transplantation in aHUS is usually not performed because of the high recurrence risk and the potential of complement mutation in the living related donor. We hypothesised that a protocol based on living kidney transplantation (which reduces ischaemia time) might allow successful kidney transplantation whilst limiting the use (and thus costs) of eculizumab. The aim of the study was to test if recurrence of aHUS can be prevented or postponed by using a protocol that minimises endothelial damage as much as possible. We describe our experience in four patients.

**MATERIALS AND METHODS**

Transplantation characteristics

In our centre approximately 110 kidney transplantations are performed each year. Currently, we use triple therapy consisting of tacrolimus 0.1 mg/kg twice daily, prednisone and mycophenolate 1000 mg twice daily as standard immunosuppression. We previously evaluated the outcome of transplantation in aHUS patients and observed both a high recurrence rate as well as a high incidence of especially acute vascular rejections in aHUS patients compared with other patients after renal transplantation. Also, we noted that the use of a standard dose of cyclosporine was related to the development of a recurrence. Overall outcome was dismal. All the above were reasons to temporarily stop transplanting aHUS patients with a high risk of recurrence.

The availability of eculizumab as rescue therapy allowed us to reconsider kidney transplantation in patients with aHUS. Living kidney donors were considered acceptable, and based on our previous experience and theoretical considerations regarding endothelial damage, we developed a protocol directed at both minimisation of risk for rejection by using quadruple immunosuppression with basiliximab induction and low-dose tacrolimus as well as reduction of risk for endothelial injury by preventing exposure to high levels of CNI, aggressive blood pressure lowering and use of statins. Steroids were not withdrawn. Details are given in box 1 and figure S1 (supplementary appendix, see http://www.njmonline.nl/all_issues.php).

**Box 1. Kidney transplantation protocol minimising recurrence of aHUS**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Goal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Living donor kidney</td>
<td>Minimise endothelial injury</td>
</tr>
<tr>
<td>Basiliximab: 20 mg day 1 and 4</td>
<td>Reduce risk of rejection, limit CNI toxicity</td>
</tr>
<tr>
<td>Tacrolimus (low dose): 0.03 mg/kg BID, trough level 5 ng/ml</td>
<td></td>
</tr>
<tr>
<td>Mycophenolate mofetil: 1000 mg BID, AUC 40-60 mg/l/h</td>
<td></td>
</tr>
<tr>
<td>Prednisone: Starting at 100 mg day 1-3, thereafter 25 mg/day and taper to 0.1 mg/kg/day at month 3 after transplantation</td>
<td></td>
</tr>
<tr>
<td>Statin</td>
<td>Endothelial protection</td>
</tr>
<tr>
<td>Blood pressure target &lt;130/80 mmHg</td>
<td>Endothelial protection</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>Limit CNI vasoconstriction</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>Limit AGE toxicity</td>
</tr>
<tr>
<td>Close patient monitoring (first three months twice weekly, second three months weekly, than 2-weekly)</td>
<td>Early detection of signs of recurrence aHUS</td>
</tr>
</tbody>
</table>

When compared with our old treatment schedule, the following differences are notable: use of living donor (vs deceased donor), use of basiliximab induction therapy (vs no induction therapy), use of low-dose tacrolimus (vs usual dose cyclosporine), and early start of ACE-inhibitors and statins.
Since 2011 this protocol has been implemented in our hospital. We contacted and screened aHUS patients linked to our centre (n=15) who would be potential transplantation candidates. We transplanted the four for which a living donor was available. While waiting for the results of transplantation in these patients, we decided not to perform transplantations with deceased donor kidneys. As per standard care all patients were informed of the risk of recurrent aHUS. Patients and related donors were aware that in case of disease recurrence treatment would consist of plasmapheresis and eculizumab.

Mutation screening
Mutational screening was performed in the coding regions of the alternative pathway genes complement factor H (CFH), complement factor I (CFI), MCP, C3, and complement factor B (CFB) by means of PCR on genomic DNA and sequence analysis. In addition, we documented the presence of three SNPs in the CFHtgt haplotype (rs3753394 [c.-331C>T], rs3753396 [c.2016A>G; p.Gln672Gln], and rs1054589 [c.2808G>T; p.Glu936Asp]) and the MCPggaac haplotype (rs2796267 [c.-652A>G], rs2796268 [c.-366A>G], rs1962149 [c.-989-78G>A], rs859705 [c.1127+638G>A], and rs7144 [c.2232C>T]), which are considered high-risk polymorphisms (table 1). One patient was screened before transplantation for the presence of autoantibodies against CFH, which was performed as described before.13

Potential living related donors were also evaluated for the presence of disease-causing mutations. A living related donor was only accepted for transplantation if a disease-causing mutation was only identified in the recipient and not in the donor.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age primary aHUS episode (years)</th>
<th>Low serum complement C3</th>
<th>Complement mutation</th>
<th>Presence of three CFH haplotype SNPs</th>
<th>Presence of MCP haplotype</th>
<th>Previous Transplant/ Age</th>
<th>Outcome first Tx</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>F</td>
<td>23</td>
<td>Yes</td>
<td>C3 p.Arg161Trp</td>
<td>rs3753394: heterozygous</td>
<td>rs2796267: heterozygous</td>
<td>NA -</td>
<td>TCD/27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C3 p.Glu1298Ala</td>
<td>rs3753396: heterozygous</td>
<td>rs2796268: heterozygous</td>
<td></td>
<td>Recurrent aHUS within 3 months</td>
</tr>
<tr>
<td>B</td>
<td>F</td>
<td>20</td>
<td>No</td>
<td>CFH p.Arg1210Cys</td>
<td>rs3753394: heterozygous</td>
<td>rs2796267: homozygous</td>
<td>LRD/27</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>rs3753396: heterozygous</td>
<td>rs2796268: homozygous</td>
<td></td>
<td>Recurrent aHUS within 3 months</td>
</tr>
<tr>
<td>C</td>
<td>F</td>
<td>50</td>
<td>No</td>
<td>C3 p.Arg161Trp</td>
<td>rs3753394: heterozygous</td>
<td>rs2796267: homozygous</td>
<td>NA</td>
<td>TCD/40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>rs3753396: heterozygous</td>
<td>rs2796268: homozygous</td>
<td></td>
<td>Recurrent aHUS within 6 months</td>
</tr>
<tr>
<td>D</td>
<td>M</td>
<td>38</td>
<td>Yes</td>
<td>C3 p.Lys65Gln</td>
<td>rs3753394: not present</td>
<td>rs2796267: heterozygous</td>
<td>LRD/40</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>rs3753396: not present</td>
<td>rs2796268: heterozygous</td>
<td></td>
<td>Recurrent aHUS within 6 months</td>
</tr>
</tbody>
</table>

CFH = factor H; LRD = living related donor; NA = not applicable. All patients were tested for mutations in CFH, CFI, MCP, C3 and CFB. 13

*C3 < 750 ng/ml. **Screened polymorphisms in CFHtgt at-risk haplotype: rs3753394 [c.-331C>T], rs3753396 [c.2016A>G; p.Gln672Gln], and rs1054589 [c.2808G>T; p.Glu936Asp]. ***Screened polymorphisms in MCPggaac haplotype: rs2796267 [c.-652A>G], rs2796268 [c.-366A>G], rs1962149 [c.-989-78G>A], rs859705 [c.1127+638G>A], and rs7144 [c.2232C>T].

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RESULTS

In 2011, we transplanted four patients with aHUS using the described protocol. In table 1 the patient characteristics are presented and in table 2 the transplantation characteristics. The individual patient characteristics were as follows.

Patient A is a 35-year-old female. In 1999 she was hospitalised with acute kidney failure, accelerated hypertension complicated by an epileptic seizure, and evidence of haemolysis and thrombocytopenia. Family history revealed aHUS in a second cousin on her father’s side. Treatment with plasma exchange and high doses of prednisone was without apparent success, although after one year of haemodialysis her kidney function recovered partially (eGFR 15 ml/min/1.73m²) allowing haemodialysis to be discontinued. Slow deterioration of kidney function necessitated renal replacement therapy and the choice for a pre-emptive kidney transplantation was made. The kidney was donated by the patient’s mother, who had no detected complement abnormalities. The CMV status required no valganciclovir prophylaxis (donor negative/recipient negative, D-/R-). The post-transplant course was not complicated by rejection or recurrence of aHUS. She suffered from hair loss due to tacrolimus treatment, rapid reversible kidney dysfunction due to hypotension, and one hospital admission because of hypertension after temporary withdrawal of the ACE-inhibitor. Currently, graft function is excellent (table 2).

Patient B is a 29-year-old female. At the age of 20 years, she was diagnosed with ESRD attributed to accelerated hypertension. In 2007 she developed acute kidney failure with clinical evidence of aHUS. Plasma exchange therapy did not improve her kidney function and the patient had to start haemodialysis. She had negative antibodies against CFH. She received the kidney of a living unrelated donor (CMV status: D-/R-). Apart from nausea due to high levels of mycophenolate mofetil in the first week after transplantation, the post-transplant course was uneventful. Patient D is a 46-year-old man. In 2003 he presented in another hospital with headache and was diagnosed with accelerated hypertension and kidney failure. The kidney biopsy showed a membranoproliferative glomerulonephritis, which could not be classified in the absence of electron microscopy. Treatment with methylprednisolone and cyclophosphamide was not effective. Two years later a living related kidney transplantation was performed. Six months later he developed acute kidney injury caused by thrombotic microangiopathy. The patient was diagnosed with aHUS (confirmed by genetic analysis) and recurrent disease in the kidney allograft. After several years he had to start haemodialysis. We performed a living unrelated kidney transplantation via our cross-over program (CMV status: D+/R+). Two weeks after the transplantation the patient had a deep venous thrombosis in his arm related to the central venous dialyses access. After removal of the catheter he was treated with low-molecular-weight heparin for three months. The patient has had no other complications to date (table 2).

DISCUSSION

Our study illustrates that kidney transplantation is feasible in patients with aHUS without using standard prophylactic therapy with eculizumab. Thus far, four patients have been successfully transplanted without evidence of recurrent disease more than 19 months after transplantation. Admittedly, the number of patients is small, and we cannot exclude that the incidence of recurrent aHUS with our protocol may be as high as 50%. Still, our experience suggests that it is acceptable to perform a randomised study to compare a protocol based on prophylactic eculizumab therapy with a protocol based on rescue eculizumab therapy.

Admittedly, the introduction of the C5-inhibitor eculizumab has dramatically changed the prognosis for aHUS patients. Many case reports have reported favourable outcome in patients with aHUS treated with eculizumab. Subsequently, eculizumab was proven beneficial in cohort studies that included patients with plasmapheresis-resistant aHUS, with a cure rate of more than 80%. In 2011 eculizumab was approved by the US Food and Drug Administration and the European Medicines Agency for the treatment of aHUS. Zuber et al. propose to use eculizumab prophylactically in moderate- and high-risk patients who undergo kidney transplantation. The authors suggest that this treatment
may be tapered after 12 months in moderate-risk patients, but should be continued in high-risk patients. Treatment according to these guidelines is associated with enormous costs and risks of suppression of complement activation.\(^5\)\(^7\) Our data suggest that prophylactic and long-term treatment with eculizumab is not inescapable in the context of kidney transplantation in adult patients with aHUS. The answer can only be found in a randomised clinical trial. However, our data provide arguments that a protocol that is based on eculizumab rescue therapy may be a reasonable option in selected patients.

Our patients represent the typical patients with aHUS. Their risk of recurrence after kidney transplantation was not low, as estimated following guidelines as recently proposed.\(^1\)\(^5\) Recurrence risk was high in patient B and D because they had a history of aHUS recurrence in a previous graft. In addition patient B had a mutation in CFH that causes diminished binding of CFH to C3b.\(^1\)\(^8\) Patient D had the p.Arg161Trp mutation in C3. This mutation also results in weakening of the C3b-CFH binding.\(^1\)\(^9\) The recurrence risk of patient A and patient C was moderate. They had the p.Arg67Trp mutation in C3. This gain-of-function mutation functionally weakens the C3b-CFH binding.\(^1\)\(^9\)\(^1\)\(^0\) In a French cohort of aHUS patients, 12 transplantations were performed in patients with gain-of-function mutations of C3 and five had a recurrence of aHUS in the graft, which results in a 42% recurrence risk.\(^1\)\(^1\)

Thus far, there is no evidence of recurrent HUS in our patients and all are doing well after a follow-up of 16-21 months. We can only speculate why outcome has been beneficial. However, we suggest that several components of our protocol, all directed at limiting endothelial cell injury (use of living donor, aggressive treatment of blood pressure and cholesterol, low dose CNI) and reducing the risk of rejection (by using basiliximab in addition to low-dose CNI, prednisone and high-dose MMF) contributed to this good outcome. Obviously, we cannot determine which factor is most critical in our protocol. Also, we cannot exclude that recurrences may occur with longer follow-up.

Zuber et al recently reported nine patients with aHUS who received a kidney transplant and were treated prophylactically with eculizumab.\(^1\)\(^2\) Outcome was excellent in all but one patient who developed arterial thrombosis immediately postoperatively. Based on these data, the authors advocate the use of eculizumab prophylactically in moderate- and high-risk patients, and they advise to continue eculizumab in high-risk patients for an undetermined period of years.

Our data provide support for the notion that prophylactic therapy with eculizumab may not be needed in aHUS. Controlled studies are needed to determine cost-effectiveness, and results of such controlled studies should be used to develop evidence-based guidelines. Certainly, our data are not unique. Øyen et al. described seven aHUS patients who were successfully transplanted without aHUS recurrence during a follow-up of more than four years after transplantation. These authors used a CNI-free regimen.\(^1\)\(^3\) Of note, genetic information was not available in these patients. Of the seven patients described by Øyen et al. four had a living donor and three patients a deceased heart-beating donor (personal communication).

The potential benefit of eculizumab as rescue therapy is illustrated in the abovementioned study of Zuber et al. who reported 13 patients who were treated with eculizumab after onset of disease recurrence. Outcome was reported as favourable, especially if treatment was started early after onset of the recurrence (within 28 days). This finding is in agreement with the observations in the clinical trials\(^1\)\(^6\) that early start of eculizumab (within one week) is related to better outcome.

We must admit that our study has limitations. There are only four patients and follow-up is relatively short, averaging 19 months. In the absence of protocol biopsies we cannot exclude subclinical thrombotic microangiopathy; however, none of the patients showed any laboratory abnormalities and their renal function is stable. Notably, our conclusions are only applicable to adults and recipients of a living donor kidney.

We propose that kidney transplantation with kidneys from living donors is feasible in patients with aHUS, and that randomised trials are justified and should be performed to compare the costs, effectiveness, and side effects of a treatment schedule that uses prophylactic eculizumab.
with a treatment schedule that merely uses eculizumab as rescue therapy. We also propose that a similar strategy should be evaluated in the setting of non-living donor transplantation.

**DISCLOSURES**

Meeting Presentation: The results were presented at the 2012 American Society of Nephrology annual meeting in San Diego, California, on 1 November, 2012.

Conflict of interest statement: NCAJ van de Kar is a member of the Alexion International Advisory Board of Ahus.

**REFERENCES**