Hemolytic Uremic Syndrome after Kidney Transplantation: Therapeutic Alternatives

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Abstract

Hemolytic uremic syndrome is a clinical entity characterized by thrombocytopenia, nonimmune hemolytic anemia, and renal impairment. Pathologic findings in kidney samples show thrombotic microangiopathy and the underlying pathogenesis is endothelial cell injury that leads to thrombotic occlusion of the arterioles and capillaries. Traditionally it was classified in two forms: typical hemolytic uremic syndrome, which occurs most frequently in children and is caused by Shiga-toxin-producing bacteria, and atypical hemolytic uremic syndrome. The latter is associated to genetic mutations regarding regulatory factors of the complement system in 50-60% of patients and has a poor prognosis, with the majority of patients developing end-stage renal disease. After kidney transplantation, hemolytic uremic syndrome can occur as a recurrent or de novo disease. Over the last years, many studies have focused their efforts on a better understanding of the pathogenic mechanisms of atypical hemolytic uremic syndrome, showing that the risk of recurrence and prognosis after kidney transplantation depends on the genetic abnormality involved. Advances in the understanding of the disease in conjunction with the recent emergence of new therapeutic options enable better strategies for prevention and treatment of atypical hemolytic uremic syndrome recurrence after kidney transplantation. This review will first summarize the complement dysregulation involved in the genesis of atypical hemolytic uremic syndrome recurrence and the factors that may injure the graft endothelium and increase the risk for developing de novo hemolytic uremic syndrome. Another part will focus on the therapeutic alternatives for hemolytic uremic syndrome after kidney transplantation according to the pathogenic mechanism involved. Finally, we will review the assessment of atypical hemolytic uremic syndrome patients before a kidney transplant and the current options to prevent its recurrence. (Trends in Transplant. 2012;6:17-27)

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Key words


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Introduction

Hemolytic uremic syndrome (HUS) is a microvascular thrombotic disorder characterized by nonimmune hemolytic anemia, thrombocytopenia, and acute renal failure. Typical HUS occurs most frequently in children and is caused by Shiga-toxin-producing bacteria (Escherichia coli). Atypical hemolytic uremic syndrome (aHUS) has a poor prognosis and progresses to end-stage renal disease (ESRD) in approximately 60% of cases. Mutations in different components of the alternative complement pathway have been found in 50-60% of aHUS cases.

After kidney transplantation, HUS can occur as a recurrent or de novo disease. The risk of recurrence depends on the pathogenesis. While typical HUS has a rate of recurrence lower than 1%, aHUS can recur after kidney transplantation in up to 80-100% of patients with complement pathway mutations.

The aim of this review is to update the available therapeutic strategies against the onset of HUS after kidney transplantation through a better understanding of the pathogenetic mechanisms that cause the disease. A second aim of this review is to highlight the therapeutic options to prevent the recurrence of aHUS after kidney transplantation.

Complement dysregulation: atypical hemolytic uremic syndrome and recurrence risk after kidney transplantation

The complement system is a major innate immune defense mechanism, with an important role in leukocyte recruitment, cell lysis, and opsonization. Complement may be activated by the classical, lectin, or alternative pathways. Recently, a close association between aHUS and mutations in the complement proteins was demonstrated, predominantly components of the alternative complement pathway (Fig. 1). Advances in genetic analysis now allow assessing the risk of aHUS recurrence after kidney transplantation and prognosis associated with specific genetic abnormalities.

Complement factor H

Complement factor H (CFH) is a polypeptide chain glycoprotein of 150 KDa synthesized in the liver and is the most important fluid phase regulator of the alternative pathway. This complement factor regulator was described in 1981 in two brothers with aHUS who did not produce CFH.

Factor H interacts with complement factor I (CFI) through the N-terminal site, constituted by short consensus repeats (SCR) 1-4, and this site gives the function to CFH as a cofactor of CFI to inhibit C3 by competing with factor B and accelerating the decay of C3 convertase. On the other hand, SCR 19-20 in C-terminal binds to glycosaminoglycans in basement membranes and endothelium, protecting against aHUS by preventing alternative complement activation. Of aHUS patients with an identified mutation on complement factor regulators, 20-30% have a mutation on CFH, with 80-100% progressing to ESRD. Most of the mutations that have been described on CFH are on SCR 19-20, which results in loss of complement regulation in renal vasculature.

Disease recurrence after transplantation has been described in around 80% of cases with almost 93% graft loss, most frequently in the first year, in more than 70 reported cases in the literature. Those with a mutation on SCR 19-20 have a higher risk of recurrence and worst prognosis.

Complement factor I

Complement factor I is a serum glycoprotein (serine protease) that is predominantly
The alternative complement pathway activation and regulation. The alternative pathway is constantly activated at a low rate by a spontaneous hydrolysis of C3. Hydrolyzed C3, combined with complement factor B, forms the initial fluid-phase C3 convertase, which cleaves C3 into C3a (anaphylatoxin) and C3b (that binds to positively charged surfaces, particularly pathogens with low expression of heparin sulfate). When C3b is bound to cell-surfaces interacts with factor B, followed by a conformational change in factor B, which is then cleaved by complement factor D to form the C3 convertase (C3bBb). This convertase cleaves more C3 molecules, initiating an amplification loop. The covalent binding to the surface without release of C3b may generate C3bBbC3b complexes with C5 convertase activity. C5 cleavage generates C5a (potent anaphylatoxin) and C5b, which participate in the formation of the membrane attack complex (C5b-C9). It can form a transmembrane channel, causing osmotic lysis of the target cell or sublytic damage associated with cell activation. In order to avoid complement hyperactivation, the alternative pathway is controlled by: complement receptors, fluid phase regulators (circulate in plasma), and membrane-bound and surface-bound complement regulators. Complement factor H – the main fluid-phase complement regulator – is able to prevent nonspecific binding of C3b to negative-charged cell surfaces, binds to complement factor I mediating the proteolytic cleavage of C3b resulting in nonfunctional iC3b and finally, competes with factor B promoting the dissociation of C3 convertase (decay-accelerating activity). Complement factor I, a serine protease, cleaves C3b in the presence of other cofactors, such as complement factor H, MCP/CD46, CR1, C4b-binding protein, and possibly thrombomodulin. Two membrane-bound proteins limit complement activation on the cell surface: DAF or CD55 by dissociating the C3 and C5 convertase and CD59 preventing C5b-9 insertion in the membrane (inhibits MAC formation). Finally, FH-related protein 1 (CFHR1) negatively regulates C5 convertase.

Casps describe 15 renal transplants received by 10 patients. Twelve renal grafts failed due to aHUS recurrence.

Membrane cofactor protein (CD46)

Membrane cofactor protein (MCP) is a single-chain transmembrane glycoprotein expressed on most human cells that binds to C3b and C4b that are deposited in the cell surface, being a cofactor for factor I, and promoting inactivation of the complement system.
Atypical HUS has been associated with widely expressed MCP, also named CD46 mutations. Membrane cofactor protein mutations were found in 9.1% of aHUS patients. This genetic predisposition led 18% of patients to ESRD, and 55% were free of dialysis.

The risk of recurrence in MCP mutations is 20%, and this is because MCP is a membrane protein and the allograft is protected by the wild-type membrane protein. Nevertheless, Fremeaux-Bacchi et al. reported recurrence after transplantation and demonstrate patchy endothelial microchimerism in the allograft and suggested that the recipient mutant MCP transferred the risk of aHUS to the graft.

**Gain-of-function mutations: complement factor B and C3**

Complement factor B (CFB) mutations lead to chronic alternative pathway activation. It is a gain-of-function mutation resulting in decreased decay of the C3bBb convertase (enhancing C3b formation) and occurs in 1-2% of aHUS patients. Recurrence of aHUS was reported in four renal transplants leading to graft failure in three aHUS patients with CFB mutations.

About 4-10% of aHUS patients have a C3 gain-of-function mutation. The mutant C3b reduces its binding to CFH and MCP, which impairs its degradation. Twelve renal transplants have been reported in aHUS patients with C3 mutations. Five of them were affected by recurrent aHUS disease (42%).

**Thrombomodulin**

Thrombomodulin is a transmembrane endothelial cell glycoprotein. Its function is to accelerate thrombin-mediated activation of protein C, which downregulates thrombin generation and suppressed clot formation. Delvaeye et al. reported that thrombomodulin binds to C3b and CFH and negatively regulates CFI. They also showed that about 5% of aHUS patients have a mutation that impairs the function of thrombomodulin. Recurrence after transplantation was reported in a single case (S924) with graft loss.

**Factor H autoantibodies**

Complement factor H antibodies have been reported since 2005. These autoantibodies are detected in 6-10% of aHUS cases and are directed against the C-terminal of CFH, and impair the binding of CFH to C3b that leads to defective factor H-dependent cell protection.

It has been associated with recurrence after transplantation, with good response to plasmapheresis or anti-CD20 monoclonal antibody (rituximab). Kavanagh et al. suggested that despite the lack of data in the literature, CFH autoantibody titers must be monitored and they recommended the administration of rituximab and plasma exchange prior to transplantation in those patients with persistent high titers.

**De novo hemolytic uremic syndrome after kidney transplantation**

The incidence of de novo HUS after renal transplantation ranges from 0.8 to 14%. The clinical presentation is highly variable, from the classic triad to only impaired kidney function without hematological manifestations. In transplant patients, a high number of factors may injure the graft endothelium and increase the risk for developing de novo HUS.

**Immunosuppression**

The most important risk factors for de novo post-renal transplantation HUS are calcineurin and mammalian target of rapamycin (mTOR) inhibitors. Calcineurin inhibitors (both cyclosporine and tacrolimus) induce renal arteriolar vasoconstriction, increase sensitivity to
vasoconstrictor agents such as endothelin-1, and decrease synthesis of vasodilator agents (prostaglandin E2, prostacyclin and nitric oxide). The concomitantly increased platelet aggregation and activating plasminogen activator (procoagulant state) may lead to the development of thrombotic microangiopathy (TMA).

The mTOR inhibitors may also act as endothelial aggressors as it has been demonstrated that sirolimus may induce downregulation of vascular endothelial growth factor (VEGF) and can induce death of endothelial progenitor. De novo post-renal transplantation HUS can occur with the use of an mTOR inhibitor alone or in combination with a calcineurin inhibitor. The combination mTOR/calcineurin inhibitor confers a pro-necrotic, proapoptotic and antiangiogenic effect on endothelial cells.

Viral infections

Some viral infections may increase the risk of developing de novo HUS in transplant patients. They include cytomegalovirus infection (associated with both recurrent forms and de novo HUS in transplant recipients), parvovirus B19, and BK polyoma virus nephritis.

Other factors

Other factors that might trigger the development of de novo HUS are: antibody mediated rejection, marginal kidneys, ischemia-reperfusion events, antiphospholipid antibodies, anticardiolipin antibodies and malignancy.

Genetic susceptibility to de novo hemolytic uremic syndrome

A study in 24 kidney transplant recipients with a history of posttransplantation de novo TMA demonstrated for the first time an association between mutations in genes coding for complement regulation proteins and de novo TMA.

Seven of 24 patients (29%) presented with CFH or CFI mutations, suggesting that these genetic abnormalities are risk factors for de novo TMA.

Therapeutic options

After identification and diagnosis, kidney transplant patients who develop HUS should be treated according to the pathogenesis of the disease.

Atypical hemolytic uremic syndrome recurrence after transplantation

Plasmatherapy

Plasmatherapy consists of two techniques: either infusion of fresh frozen plasma (FFP), or plasma exchange and restitution with FFP. These techniques were firstly used in 1977 on thrombotic thrombocytopenic purpura with generally good results. However, it was not possible to achieve the same percentage of favorable response in HUS until the difference between typical and atypical HUS was described. Plasma infusion leads to the addition of non-mutated proteins that allows complement regulation; moreover, plasma exchange can remove the mutated factors and may also remove some proteins that can trigger complement activation, such as cytokines.

Descriptions in the literature of plasmatherapy used for CFH mutations led us to conclude that it could induce partial (49.5%) or complete remission (17.5%), but up to 70% of patients die, require dialysis, or have chronic renal insufficiency. If used, it has to be initiated early, with caution when there is infection or inflammatory responses to enhanced therapy. From these reports it seems that plasma exchange can be superior to plasma infusion for treatment and prevention.

In respect to CFI, the reports describe partial response in more than 50% of cases.
Nevertheless, almost all patients developed ESRD. It seems that almost 50% of patients with CFI mutations may have other additional complement factor mutations that worsen the prognosis, leaving those with just a CFI mutation with a better prognosis13,66.

One cannot expect a beneficial effect over plasmatherapy on MCP or thrombomodulin mutations due to the nature of membrane protein. Retrospective studies of French and Italian registries showed no differences in the outcome when comparing plasma exchange or plasma infusion with no treatment and as a preventive tool, as was described later6,66,67.

It is not clear whether plasmatherapy can be useful in gain-of-function mutations, and Loirat, et al. suggest that more frequent plasma exchange might be needed to maintain a low or null activation of complement66.

Loirat, et al.66 give empirical guidelines based on literature reports and recommend initiating plasmatherapy within the first 24 hours of presentation of symptoms and laboratory alterations, with plasma exchange as first-line therapy with a 1.5 plasma volume exchange with FFP. If this is not possible, they recommend the use of plasma infusion at a dose of 10-20 ml/kg. This is true for CFH, CFI, C3, and CFB mutations. If MCP or thrombomodulin are confirmed, then plasmatherapy can be suspended; otherwise, they recommend permanent therapy66.

**Eculizumab**

Eculizumab is a humanized monoclonal antibody against complement factor 5 (C5). By blocking the cleavage of C5, generation of prothrombotic C5a and the formation of membrane attack complex (C5b-9) is prevented. Eculizumab was first approved for the treatment of paroxysmal nocturnal hemoglobinuria and in 2011 was approved by the FDA for all patients with aHUS68.

Clinical cases reported with the use of eculizumab in aHUS recurrence after kidney transplantation showed that the inhibition of microangiopathic hemolytic processes (normalization of platelet count, lactate dehydrogenase, and haptoglobin) occurred in all reported patients within a short period of time, but renal function recovery was variable69-73. Some authors suggest that renal recovery may depend on early administration of eculizumab before significant and permanent renal damage occurs70,73. One of the above patients had a recurrence of aHUS after a six-day delay of eculizumab infusion70.

Recently, Zuber, et al.74 extended these experiences with a series of 13 renal transplant patients treated with eculizumab after post-transplant aHUS recurrence. In all of them hematological signs of hemolysis rapidly returned to normal range and renal function improved significantly during the first three months. They observed relapses in two patients who received a single-dose and two patients who delayed (6-8 days) the eculizumab infusion. Among the 11 patients who maintained on eculizumab, the interval between the onset of the aHUS recurrence and anti-C5 initiation determined the extent of recovery of renal function.

Taken together, the published data suggest that eculizumab is an effective and safe therapy, and should be recommended as first-line treatment of posttransplant aHUS recurrence. Nevertheless, the optimal regimen (timing and dosages) either for prophylaxis or treatment need further study. Ongoing clinical trials with an estimated study completion date in December 2012 will give us more details on eculizumab efficacy and safety75,76.

**New therapies**

The advent of new therapies currently under investigation is promising for some targets. Complement regulatory proteins have been synthesized and may be a future therapeutic option, like concentrated CFH for aHUS patients with CFH mutations77. Other complement regulatory
proteins have been synthesized, such as decay accelerating factor and MCP, but the effectiveness on complement inhibition was lower than the parental molecules. Attempts to verify other alternatives, such as TT130 molecule directed to inhibit C3 and TA106 (a molecule directed against factor B) have shown results in animal models, without having been tested yet in humans, as well as monoclonal antibodies against factor B. Molecules described in aHUS development like thrombomodulin can be of possible use, as was shown by Sakai, et al., using recombinant human soluble thrombomodulin, a molecule already approved for intravascular disseminated coagulopathy in Japan, to gain control of TMA after hematopoietic stem cell transplantation. The achievement of new molecules directed against targets in complement modulation may be of interest to obtain control of aHUS.

**De novo hemolytic uremic syndrome after transplantation**

**Avoiding triggers**

A first step should be to identify possible triggers and to try to eliminate them. Therefore, it seems logical to use calcineurin inhibitor-free protocols to avoid ischemia-related endothelial damage. However, the benefit of these therapies remains controversial. While some studies have shown benefits of avoidance of calcineurin inhibitors in de novo posttransplant HUS, others have reported no benefits. Furthermore, the most extensive alternative to calcineurin inhibitors is an mTOR-based regimen, but sirolimus can also induce endothelial damage. A promising alternative could be a maintenance immunosuppressive regime based on belatacept. Ashman, et al. reported a single case of de novo HUS after living-donor kidney transplant on cyclosporin, with recurrence after switch to tacrolimus first, and sirolimus afterwards. Finally, belatacept has been safely introduced as maintenance immunosuppression in combination with prednisone and azathioprine, showing successful resolution of TMA.

**Plasmatherapy**

Plasma exchange used along with discontinuation of calcineurin inhibitors has shown durable remission in 80% of patients with de novo posttransplant HUS (23 of 29 patients). Therefore, plasma exchange therapy is a mainstay in the treatment of this disease.

**Eculizumab**

Recently, Chandran, et al. reported a case of successful treatment with eculizumab of de novo TMA associated to acute antibody rejection in a pancreas/kidney transplant. Wilson, et al. also reported a case of de novo TMA in a pancreas/kidney transplant treated unsuccesfully with plasma exchange that rapidly responded to eculizumab treatment.

**Assessment of atypical hemolytic uremic syndrome before transplantation**

**Investigations**

Before a patient with ESRD secondary to aHUS is included in a waiting list for transplantation, a complete genetic assay must be done in order to define the strategies for the management of the future transplant patient (Table 1).

**Prophylactic strategies**

The presence of gene mutations conferring a high risk of recurrence and graft lost (CFH, CFI, C3 and CFB) would be an obvious reason for prophylactic intervention (PE, eculizumab) or liver-kidney transplantation. Other abnormalities with low risk of aHUS recurrence should be considered on an individual basis (MCP mutations or negative CFH antibodies).
Preemptive plasma exchange

From the experience reported in the literature, it seems that preemptive plasma exchange could be beneficial for CFH, CFI mutations and anti CFH autoantibodies and it is not clear if this could have any benefit on gain-of-function mutations, and possibly there is no benefit on MCP or thrombomodulin mutations.6,66.

Liver/kidney transplantation

Due to the synthesis of CFH and CFI in the liver, the rationale of a liver/kidney transplant was logical. However, Remuzzi, et al. reported two cases of aHUS because of CFH mutations that underwent liver/kidney transplants with fatal outcomes for both of them.86,87. Additionally, Cheong, et al. reported in 2004 an attempt of auxiliary partial orthotopic liver transplantation in a 30-month-old child with low CFH levels and hemolytic anemia, but the child died 11 months later from posttransplant lymphoproliferative disorder.88.

In spite of these three initial attempts, in 2009, Saland, et al. proposed treatment guidelines on liver/kidney transplantation for aHUS based on several successful reports of liver/kidney transplants in patients with CFH and CFI mutations. It is not clear yet if other types of mutations, like C3 or CFB, can be treated with this alternative, given the extrahepatic site of production. They recommend combined liver/kidney transplantation in patients with aHUS and ESRD with CFH or CFI mutations. This treatment must be accompanied by plasma exchange (1.5 vol. FFP in 4-6 hours) and intraoperative plasma infusion (10-20 ml/kg of FFP infused after native liver is explanted), and after the transplantation, low molecular weight heparin at prophylactic doses and aspirin (2 mg/kg/day up to 80 mg/day) for three months. Guidelines make no specific recommendations as to which immunosuppression to use. However, they recommend liver transplantation alone if there is no clinical kidney function deterioration, performing a kidney biopsy first to evaluate the extent of kidney histological alterations. They also recommend isolated kidney transplantation in those patients with no evidence of CFH, CFI, C3 and CFB mutations, with aHUS due to MCP mutation or FH autoantibodies.8,89.

Prophylactic eculizumab

Primary prophylaxis prior to kidney transplantation has been first described by Zimmerhackl, et al. in a CFH-deficient four-year-old patient, with eculizumab every two weeks for 12 months, with no documented recurrence. A second case was reported by Weitz, et al. in a renal transplant in a seven-year-old child with a CFH mutation. They used eculizumab prior to transplantation and then every two weeks for a seven-month follow-up period, without allograft rejection and only reporting a polyoma virus infection that resolved after cidofovir treatment. Recently, Zuber et al. collected these positive experiences along with seven other cases who received prophylactic eculizumab to prevent posttransplant aHUS recurrence. Of the nine patients, eight experienced a recurrence-free

| Table 1. Investigations of the complement system in atypical hemolytic uremic syndrome patients |
|-----------------|-----------------|
| Plasmatic levels of complement factors |
| Complement C3 |
| Complement C4 |
| Complement factor H |
| Complement factor I |
| Complement factor B |
| Genetic study (genotyping) |
| Complement factor H |
| Complement factor I |
| Membrane cofactor protein |
| Complement C3 |
| Complement factor B |
| Thrombomodulin |
| Screening for autoantibodies against complement factor H (CFH) |
posttransplant course (one lost her graft because arterial thrombosis) after a median follow-up of 14.5 months.

Despite the limited size, these data suggest that prophylactic eculizumab could be a suitable strategy in aHUS patients with high risk of recurrence after renal transplantation.

Living-donor kidney transplant

Despite the high risk of aHUS recurrence after a cadaveric-donor kidney transplant, the introduction of new effective prophylactic strategies (plasma exchange, liver/kidney transplantation, eculizumab) make a deceased-donor kidney transplant a good choice for aHUS patients. Nevertheless, living-related donor kidney transplantation in aHUS patients has been categorically contraindicated in the literature due to the significant and unacceptable risk of aHUS recurrence and also for the risk to the donor. Four case reports described aHUS presentation in the donors within the first year of donation.

However, our knowledge on complement factor mutations, hot spots or single nucleotide polymorphisms, and genetic screening is today more complete than 10 years ago. If living-related donor kidney transplantation is the only choice, a complete and thorough evaluation of the patient and donor must be done prior to transplantation. Living-related donor kidney transplantation should only be considered if aHUS mutations are identified in the recipient and are not present in the donor (excluding the presence of genetic susceptibility to aHUS). Every case should be considered with caution and complete information about the risks should be explained to both recipient and donor.

Conclusions

Atypical hemolytic uremic syndrome is a disease that is related to mutations in genes that encode molecules regulating the complement alternative pathway. To date, many descriptions have been made on the loci of these mutations, providing knowledge about the regulation of complement pathway. Most of these mutations have a poor prognosis and lead to progression to ESRD. Treatment with an isolated kidney or combined liver/kidney transplantation seems a good alternative, nevertheless, depending on which complement factor is mutated. There will be more or less risk of recurrence, so a thorough genetic evaluation must be done in order to include patients in a waiting list. Moreover, pre-emptive therapy with plasmapheresis and/or eculizumab is recommended; however, the design and outcomes of new therapies may give us new tools to prevent and treat recurrences.

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References

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