New insights into postrenal transplant hemolytic uremic syndrome

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Abstract | After renal transplantation, hemolytic uremic syndrome (HUS) may occur either as a recurrent or *de novo* form. Over the past decade, much effort has been devoted to elucidating the pathogenesis of atypical HUS (aHUS). Approximately 60–70% patients with aHUS have mutations in regulatory factors of the complement system or antibodies against complement factor H. The risk of post-transplant recurrence of aHUS depends on the genetic abnormality involved, and ranges from 15% to 20% in patients with mutations in the gene that encodes membrane cofactor protein and from 50% to 100% in patients with mutations in the genes that encode circulating regulators of complement. Given the poor outcomes associated with recurrence, isolated renal transplantation had been contraindicated in patients at high risk of aHUS recurrence. However, emerging therapies, including pre-emptive plasma therapy and anti-C5 component monoclonal antibody (eculizumab) treatment have provided promising results and should further limit indications for the risky procedure of combined liver–kidney transplantation. Studies from the past 2 years have demonstrated genetic abnormalities in complement regulators in 30% of renal transplant recipients who experienced *de novo* HUS after renal transplantation. This finding suggests that the burden of endothelial injury in a post-transplantation setting may trigger *de novo* HUS in the presence of mild genetic susceptibility to HUS.

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Introduction

Postrenal transplant hemolytic uremic syndrome (HUS) can occur as a recurrent or *de novo* disease. In patients with HUS-related end-stage renal disease (ESRD), the risk of HUS recurrence after transplantation largely depends on the pathogenetic mechanisms involved. For instance, HUS caused by infection with Shiga-toxin-producing *Escherichia coli* or *Streptococcus pneumoniae* (that is, typical HUS), has a low rate of post-transplant recurrence (<1%).[1–3] By contrast, atypical HUS (aHUS), which accounts for 10% of juvenile cases and the majority of adult cases, generally has a much higher rate of recurrence after transplantation.[3–6] Indeed, post-transplant recurrence of aHUS can occur in 80–100% of patients who have mutations that cause dysregulation of the alternative complement pathway.[3–6] This observation stresses that the mechanistic dissection of aHUS will provide critical tools for the improved management of patients with HUS. This Review aims to shed light on recent advances in the understanding of the pathogenesis of postrenal transplant HUS. Diagnostic and therapeutic considerations with regard to renal transplantation are also discussed and data showing that *de novo* and recurrent forms of post-transplant HUS share common pathogenic mechanisms are described.

Competing interests
J. Zuber, V. Frémeaux-Bacchi and C. Legendre declare an association with the following company: Alexion Pharmaceuticals. C. Loirat declares an association with the following companies: Alexion Pharmaceuticals and LFB Biotechnologies. See the article online for full details of the relationships. M. Le Quintrec and R. Sberro-Soussan declare no competing interests.

Defective complement regulation in aHUS

Complement dysregulation and renal disease

To date, genetic abnormalities identified in patients with aHUS have predominantly involved components of the alternative complement pathway (Figure 1, Box 1). Experimental and clinical data highlight the critical role of complement regulation on the endothelium in preventing HUS. The complete absence of complement factor H (CFH) in *Cfh*-deficient mice is associated with the uncontrolled activation of fluid-phase complement C3 (C3), which leads to the development of membranoproliferative glomerulonephritis (MPGN).[7] By contrast, transgenic mice that express a truncated version of CFH, which is devoid of the endothelium-binding C-terminus, maintain regulation of plasma levels of C3 but develop HUS.[8] This finding is consistent with the clinical observation that homozygous mutations in *CFH* that are associated with quantitative deficits in CFH are primarily found in patients with MPGN,[9] whereas most heterozygous mutations associated with HUS are located within the C-terminal region of *CFH*.[3]

Frequency of complement mutations in aHUS

Intensive investigation over the past decade has yielded tremendous insight into the frequency of mutations in

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Key points

- Mutations in genes that encode components of the alternative complement pathway (for example, *CFH*, *CFI*, *MCP*, *C3* and *CFB*) and anti-complement factor H antibodies have been identified in 60–70% of patients with atypical hemolytic uremic syndrome (aHUS)
- Mutations in genes that encode regulatory factors of the complement system (for example, *CFH*, *CFI* and *MCP*) have been identified in 30% of cases of *de novo* post-transplant hemolytic uremic syndrome (HUS)
- The risk of aHUS recurrence after renal transplantation varies according to which factor is mutated: the risk is low (~15%) for mutations in membrane cofactor protein and high (~80%) for mutations in circulating proteins
- Post-transplant recurrence of aHUS is associated with a poor outcome; therefore living-donor renal transplantation is currently not recommended in the setting of aHUS
- The multitude of endothelial aggressors in the post-transplant setting may trigger *de novo* HUS in patients with mild genetic susceptibility to HUS
- Innovative therapeutic avenues, including pre-emptive plasma therapy and anti-C5 antibody therapy are extremely promising for the prevention or cure of recurrent aHUS, and should limit the indications for combined liver–kidney transplantation

regulatory components of the complement system in aHUS populations (Table 1).[3] Data from the International Registry of Recurrent and Familial HUS/Thrombotic Thrombocytopenic Purpura[4,5] and from national cohorts of European countries[4,10,11] have been particularly useful in this regard.

Mutations in genes encoding CFH,[12,13] complement factor I (CFI),[11,14–16] or membrane cofactor protein (MCP)[17–19] have been found in 20–30%, 2–12% and 10–15% of patients from these registries, respectively. Gain-of-function mutations in genes that encode complement factor B (CFB)[20,21] and C3[22] have been identified in 1–2% and 10% of aHUS patients, respectively. In the past year, mutations in *THBD*, which encodes thrombomodulin, were identified in 5% of patients with aHUS.[3,23] Finally, anti-CFH antibodies are readily detected in 5–10% of patients with aHUS.[24–28]

aHUS: a complex polygenic disease

Most of the mutations mentioned above have an incomplete penetrance. The penetrance for mutations in *CFH*, *CFI*, *MCP*, *C3*, *CFB* and *THBD* is estimated to be 50%.[3,20,22,23,29–31] Of note, at least 10% of affected patients have a combination of two mutations,[5,11,32–34] which suggests that aHUS may result from the additive effects of several genetic factors. In addition to mutations, various polymorphisms are associated with aHUS, including those in genes encoding C4b-binding protein (C4b–BP),[35] CFH-related protein 1 (CFHR1),[27] MCP,[29,31] and CFH.[29,31,36,37] Such genetic polymorphisms may constitute a minor degree of susceptibility to aHUS and could influence the development and severity of disease.[11,34] Furthermore, disease-affected members of families with inherited mutation-related aHUS frequently harbor additional genetic susceptibility factors for HUS, whereas healthy carriers of the mutation do not.[5,10,30,32–34,36,38] Nevertheless, the influence of each polymorphism and the effect of gene–gene interactions may be difficult to assess in a given individual because the penetrance and expression of aHUS can also be influenced by epigenetic and environmental factors. The FH–HUS Mutation Database[39] might be useful in evaluating the functional impact of newly identified genetic abnormalities in *CFH*, *CFI* and *MCP*.[37]

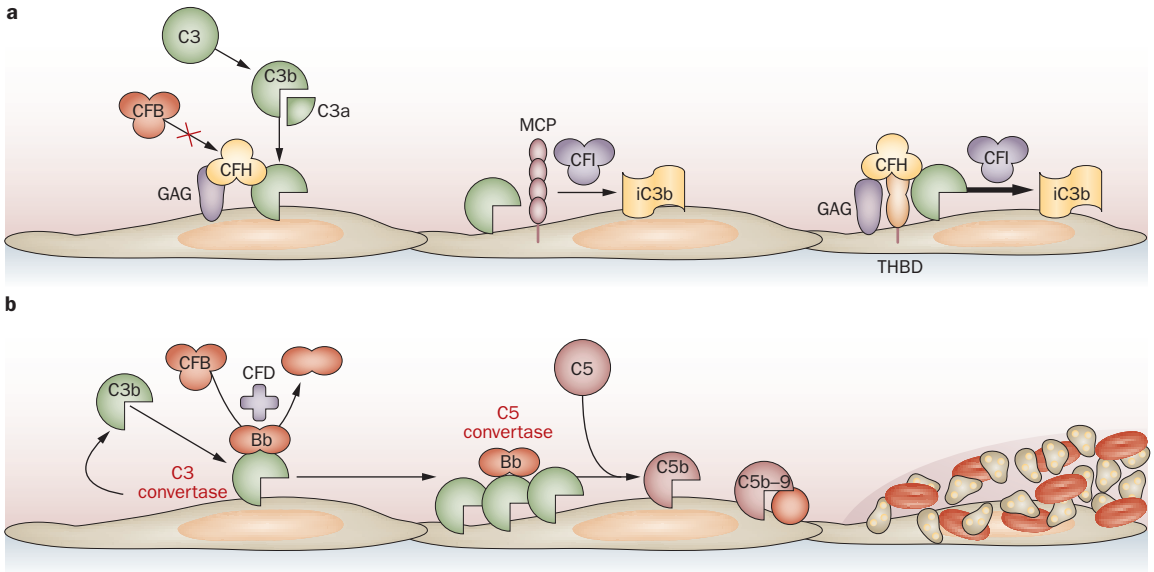

Figure 1 | Regulated and deregulated activation of the alternative complement pathway. **a** | CFH competes with CFB to bind C3b, which hampers the generation of C3 convertase. CFH binds to glycosaminoglycans on the endothelial surface and factors, such as MCP, can act as a cofactor for the CFI-mediated cleavage of C3b to generate iC3b. THBD binds to C3b and CFH and might accelerate the CFI-mediated inactivation of C3. **b** | Uncontrolled activation of the alternative complement pathway leads to the generation of the membrane-attack complex (C5b–9) through the actions of CFB, CFD and through the generation of C3 convertase and C5 convertase (Box 1). The resulting injury and activation of endothelial cells initiates a microangiopathic thrombotic process. Abbreviations: CFB/D/H/I, complement factor B/D/H/I; GAG, glycosaminoglycan; MCP, membrane cofactor protein; THBD, thrombomodulin.

Evolving boundaries of knowledge

Although mutations in complement regulatory factors or anti-CFH antibodies have been documented in 60–70% of patients with aHUS (Table 1), several questions about the pathogenetics of this disease remain unanswered. First, unidentified genetic abnormalities that contribute to the disease may exist. Patients with low C3 levels or with a family history of aHUS, in whom mutations in *CFH*, *CFI*, *MCP*, and *C3*[10,40] have been excluded, suggest that this field of investigation is not completely defined. Second, the clinical relevance of genetic polymorphisms in the genes encoding CFH-related proteins remains a matter of active investigation. A high degree of homology exists between *CFH* and five genes (*CFHR1–5*), which are located downstream of *CFH* in a head-to-tail arrangement. The sequence homology between these contiguous genes promotes genomic rearrangements and genetic polymorphisms through gene conversion and nonhomologous recombination.[25,27,38,41,42] Rarely, such rearrangements result in the formation of a hybrid *CFH/CFHR1* gene, which encodes a nonfunctioning protein. The association of such nonfunctioning proteins with aHUS is well established.[42] More frequently, deletions and allelic polymorphisms occur.[25,27,28,38,43] One study found a large deletion encompassing *CFHR1* and *CFHR3* (*CFHR1–CFHR3*del) to be associated with aHUS, suggesting a critical role for CFHR1 and CFHR3 in regulating complement activation.[43] However, four studies subsequently showed that the increased frequency of the *CFHR1–CFHR3*del mutation in aHUS versus healthy populations (14–23% versus 3–8%) resulted primarily from the strong association between *CFHR1* deletion and anti-CFH antibody levels.[25,27,28,38] On the other hand, *CFHR1* deficiency alone, regardless of the presence of anti-CFH antibody, can modulate the severity of aHUS associated with another mutation.[11] Data from a 2009 study support a role for CFHR1 in the regulation of complement activation by inhibiting C5 convertase activity.[44] Nevertheless, the mechanism by which the complete absence of CFHR1 triggers self-reactivity against CFH is currently unknown and largely speculative.[27] Finally, another 2009 study found a new allelic variant of *CFHR1* (*CFHR1*B*) to be associated with aHUS when present in the homozygous state.[27]

Box 1 | The alternative pathway of the complement system

The complement system is a proteolytic cascade that comprises >30 circulating and membrane-bound proteins. Complement is activated by three pathways, the classical, alternative and lectin pathways, which converge to form the membrane attack complex (MAC).[130] The alternative pathway is constitutively active at low levels through spontaneous hydrolysis of complement C3 (C3). Hydrolyzed C3, combined with complement factor B (CFB), forms the intermediate C3 convertase, which cleaves C3 to produce C3a and C3b. C3b binds to pathogens and host cell membranes, particularly those with low expression levels of cell-surface heparan sulfate. C3b is deposited onto the activating surface and binds CFB, which is then cleaved by complement factor D to form the C3 convertase C3bBb. The latter cleaves C3, initiating an amplification loop, and leading to the formation of C5 convertase C3bBb(C3b)n. The C5b component, generated by C5 cleavage, participates in the assembly of the MAC C5b–9. A number of membrane-anchored and circulating regulatory proteins prevent activation of complement on both resting and activated endothelial cells. In the setting of endothelial injury, the induction of heparanase and the parallel loss of heparan sulfate promote the formation of C3 convertase.[131] This phenomenon may be critically enhanced by a defective complement regulatory system. Complement factor I, a serine protease, cleaves and inactivates C3b to form iC3b in the presence of cofactors, including MCP, complement factor H (CFH), C4b-binding protein (C4b–BP), and possibly thrombomodulin.[23] The competition between CFH and CFB binding to C3b also limits formation of C3 convertase. In addition, CD55 (also known as DAF) and CD59, two membrane-bound proteins, further limit complement activation on the cell surface. CD55 promotes the dissociation of the C3 and C5 convertase complex, whereas CD59 inhibits MAC formation.

Table 1 | Risk of aHUS recurrence according to the implicated genetic abnormality

Gene	Protein location	Functional impact	Mutation frequency in aHUS (%)	Recurrence frequency after transplantation (%)
Mutation				
CFH	Plasma	Loss	20–30	75–90
CFI	Plasma	Loss	2–12	45–80
CFB	Plasma	Gain	1–2	100
C3	Plasma	Gain	5–10	40–70
MCP	Membrane	Loss	10–15	15–20
THBD	Membrane	Loss	5	1 case
Genetic polymorphism (frequency in control populations)				
Homozygous *CFHR1*del (3–8%)	Circulating	Undetermined	14–23 (>90% in patients with anti-CFH antibodies)	NA

Abbreviations: aHUS, atypical hemolytic uremic syndrome; C3, complement C3; CFB/H/I, complement factor B/H/I; MCP, membrane cofactor protein; NA, not available; THBD, thrombomodulin.

Complement and post-transplant aHUS

Before the genetic analysis of complement regulators was possible, aHUS was noted to recur in approximately 20% of pediatric patients and 50–60% of adult patients after renal transplantation.[2,45–47] Several studies over the past few years have, however, now assessed the risk of aHUS recurrence and prognosis associated with specific genetic abnormalities (Table 1).[4,5,10,40,48]

Mutations in *CFH* and *CFI*

A 2006 meta-analysis of 36 renal transplantations in 27 patients with aHUS associated with a mutation in *CFH* estimated the overall recurrence rate to be 73.7%.[4] Recurrence of aHUS was associated with poor prognosis, leading to graft loss in 93% of patients, typically within the first year of transplantation (86%).[4] In two French case series, the frequency of aHUS recurrence in carriers of *CFH* mutations was 80% in five children[10] and 75% in 16 adults,[40] who had received six and 17 renal transplants, respectively. Recurrence of aHUS was responsible for graft loss in 60% of these patients.[10,40] Interestingly, the location of the mutation within *CFH* could also influence the risk of recurrence.[49] Mutations that resided in the short consensus repeat (SCR) domains SCR1–15 were associated with a lower risk of recurrence than those lying within SCR19 and SCR20 (44% versus 76%).[4,49] Thus, mutations located within the C-terminal domain (SCR19 and SCR20) of *CFH* were not only those predominantly identified in patients with aHUS,[3]

but were also associated with the worst prognosis.[4,49] This observation supports the critical role of the CFH C-terminal domain in binding the endothelium and protecting against aHUS.[8]

The majority of anti-CFH antibodies bind and inhibit the activity of C-terminal SCR domains[26,28,50] a finding that may lead one to expect a link between high levels of anti-CFH antibody and post-transplant recurrence of aHUS. This assumption is supported by data from a 2010 *in vitro* study, which demonstrated that enhanced activation of complement correlated with increased titers of anti-CFH antibody.[50] Nevertheless, the risk of post-transplant aHUS recurrence in patients with anti-CFH antibodies is not well understood, since available reports only describe a total of 12 renal transplants in eight patients.[3,27,28,51–53] The assessment of risk is further complicated by the 2010 finding that almost 40% of patients with anti-CFH antibodies in a single cohort also carried a mutation in genes encoding complement regulatory factors, including *CFH*, *CFI*, *MCP* and *C3*.[28] A reduction in levels of anti-CFH antibodies with treatment, including plasmapheresis and rituximab, enabled successful renal transplantation in these patients, suggesting that high anti-CFH antibody levels positively correlate with risk of aHUS recurrence.[51,52] On the other hand, publications from the past 2 years have reported that recurrence-free transplantation is achievable in patients with anti-CFH antibodies despite the absence of any specific treatment.[28,52,53] However, given the lack of pretransplant screening for anti-CFH antibodies in these latter publications,[28,53] it seems reasonable to recommend that titers of anti-CFH antibody are regularly monitored in an attempt to evaluate the risk of post-transplant aHUS recurrence.

Renal transplantation in aHUS patients with *CFI* mutations is also associated with a high rate of aHUS recurrence and poor prognosis.[3,5,10,14–16,54–56] Available reports describe 10 patients, who received 15 renal transplants.[3,5,10,14–16,54–56] 12 of 15 (80%) transplants consecutively failed because of aHUS recurrence. However, a 2009 study showed that more than half of the *CFI*-mutation carriers harbored an additional genetic susceptibility factor for aHUS and that those with an isolated *CFI* mutation had improved kidney survival over those with additional mutations.[11] The post-transplant risk of aHUS recurrence for patients with an isolated *CFI* mutation should therefore be reassessed in light of this finding.

Mutations in *MCP* and *THBD*

MCP is a transmembrane protein; its expression within the graft endothelium is driven by the donor genome. Renal transplant recipients who have mutations in *MCP* are expected to have a low risk of aHUS recurrence and a poor response to plasma therapy.[57] The prognosis of patients with *MCP* mutations after renal transplantation is consistently much better than that of patients with mutations in *CFH* and *CFI*.[5,10,18,19,57] Of 15 renal transplantations performed in 13 patients with aHUS who had mutations in *MCP*,[5,10,18,19,40,57] only three recurrences were reported.[19,40,58] Two mechanisms have been implicated to explain recurrence in patients with *MCP* mutations. The first possibility is that graft endothelial cells may be partially replaced by circulating endothelial cells of donor origin that express the mutated *MCP*.[58] Alternatively, the *MCP* mutation might be associated with another unknown genetic abnormality that could contribute to disease susceptibility. Indeed, a low C3 level was found in one of the patients described above.[19,40]

Data on the post-transplant recurrence of aHUS in patients with mutations in *THBD* are too limited to draw any firm conclusions about the risk of recurrence associated with *THBD* mutations. In the available study, which describes seven patients with *THBD* mutations, one individual experienced post-transplant recurrence of aHUS, despite the fact that the graft should have expressed the nonmutant protein.[23] As functional THBD exists in a soluble form, we hypothesize that *THBD* mutations could be associated with an increased rate of post-transplant recurrence compared with that associated with mutations in membrane-bound MCP.[59]

Mutations in *C3* and *CFB*

Recurrence of aHUS was reported in five of 12 renal transplant recipients (42%) with mutations in *C3*.[3,22] It is tempting to speculate that production of nonmutant C3 by the graft might account for the reduced recurrence rate compared with that observed after transplantation in individuals with mutations in *CFH* or *CFI*.[60,61] Four renal transplantations have been reported in three aHUS patients with *CFB* mutations.[20,21] All four transplants failed because of aHUS recurrence.[20,21]

De novo aHUS after transplantation

Epidemiological and clinical features

De novo HUS occurs in 1–5% of renal transplant recipients, most frequently within the first 3 months after transplantation.[62,63] Although one study of *de novo* post-transplant HUS (which used data based on Medicare claims registered in the US Renal Data System) reported that the incidence of *de novo* post-transplant HUS was low (0.8%),[63] another series (which used a liberal biopsy policy) reported the incidence of *de novo* post-transplant HUS to be 14% in patients who experienced a rise in serum creatinine level of ≥44.2 μmol/l above baseline.[64] Notably, of 26 patients in the latter study who had biopsy-proven thrombotic microangiopathy (TMA) after transplantation, only two exhibited hematological features suggestive of HUS, including thrombocytopenia and/or hemolytic anemia.[64] This finding underscores the rarity of frank cytopenia in the setting of *de novo* post-transplant HUS. We believe that the frequency of post-transplant TMA is best assessed by performing protocol biopsies at predetermined times after transplantation, regardless of graft function.[65–67] We also believe that as the incidence of *de novo* HUS greatly depends on the definition used, the terms HUS and TMA should be used to describe the features of disease as assessed clinically and histologically, respectively, according to the commonly accepted definitions.[68]

Environmental triggers

Renal transplantation combines several factors that may act synergistically to injure the graft endothelium. Such an injury might serve as a trigger event, initiating the development of HUS. The endothelium might be injured by alloimmune responses, especially those mediated by antihuman leukocyte antigen antibodies,[69–71] antiphospholipid antibodies,[72] ischemia–reperfusion events,[73] immunosuppressive drugs,[64,66] and viral infections.[74–77]

Immunosuppressive drugs

Although both ciclosporin and tacrolimus are associated with nephrotoxicity, including TMA,[66,68,78–80] maintenance immunosuppressive regimens that are currently used in renal transplant recipients commonly include one of these two calcineurin inhibitors.[81] Calcineurin inhibitor-induced nephrotoxicity primarily results from dose-dependent renal arteriolar vasoconstriction,[82] owing to the enhanced production of vasoconstrictive factors, particularly endothelin-1 and angiotensin II.[78,83] In addition, calcineurin inhibitors can cause increased sensitivity to endothelin-1[78,84] and the decreased synthesis of vasodilatatory factors, such as prostacyclin, prostaglandin E2 and nitric oxide.[78,80,84] Moreover, calcineurin inhibitors might promote a procoagulant state by enhancing platelet aggregation and activating plasminogen activator.[80] Both ciclosporin and tacrolimus have consistently been associated with increased plasma indicators of endothelial injury, such as thrombomodulin, von Willebrand factor, and circulating endothelial cells.[66,68,85] Prolonged calcineurin inhibitor-related ischemia is believed to induce endothelial damage and initiate the pathogenic processes involved in the development of TMA.[80]

In an attempt to limit the risk of calcineurin-inhibitor-associated nephrotoxicity, use of the mTOR inhibitors sirolimus and everolimus has been proposed. These drugs could either substitute for the calcineurin inhibitor or be combined with a low dose of calcineurin inhibitor. However, *de novo* post-transplant HUS has been reported both with use of an mTOR inhibitor alone and in combination therapy.[80,86–88] Indeed sirolimus was identified as an independent risk factor for *de novo* post-transplant HUS.[63] This clinical observation contrasts with the experimental findings that sirolimus reduced levels of endothelin-1, preserved nitric oxide synthesis and did not impair endothelium-dependent vasorelaxation.[84] Sirolimus has, however, been shown to inhibit the secretion of local VEGF,[88] which may be sufficient to trigger the development of TMA.[89] In addition, sirolimus can induce death of endothelial progenitor cells.[90] Thus, sirolimus interferes with endothelial cell biology, which leads to reduced repair capacity after injury.[80] The combined administration of a calcineurin inhibitor and sirolimus confers the pronecrotic effect of the former and proapoptotic effect of the latter and is associated with a 16-fold higher risk of post-transplant HUS compared with a combined calcineurin inhibitor and mycophenolate mofetil regimen.[86,87]

Viral infections

Viral infections, to which patients on immunosuppression are susceptible, have been implicated in the pathogenesis of *de novo* HUS in organ transplant recipients. Cytomegalovirus (CMV) infection has been associated with both *de novo*[74] and recurrent forms[75] of post-transplant HUS. CMV replication has been shown to induce primitive chronic endothelial dysfunction,[91] increase circulating levels of endothelial biomarkers, such as endothelial microparticles,[85] and trigger CMV-specific cytotoxic immune responses, which target endothelial cells.[92] Parvovirus B19 and polyoma BK virus infection have also been associated with *de novo* HUS in renal transplant recipients.[76,77,93]

Genetic susceptibility

A 2008 study by Le Quintrec *et al.*[33] assessed whether mutations in genes that encode regulators of the complement system increase the susceptibility of renal transplant recipients with non HUS-related ESRD to *de novo* HUS. Strikingly, a mutation in *CFH* and/or *CFI* was found in seven of 24 (29%) patients with *de novo* post-transplant HUS,[33] including in four patients in whom a nephropathy other than HUS had been proven by kidney biopsy. Importantly, all 24 patients exhibited acute kidney injury and/or hematological features of TMA.[33] By contrast, the same research group failed to identify any genetic abnormality in the complement pathway in 10 patients with subclinical histological lesions of TMA (Le Quintrec, M. unpublished data). As genetic investigations were limited to the sequencing of *CFH*, *CFI* and *MCP*, it is possible that this study might have underestimated the frequency of defects in the regulation of the alternative complement pathway. We have proposed that genetic susceptibility could predispose certain individuals to the development of aHUS involving both naive and transplanted kidneys.[33] However, in the post-transplant setting, the multitude of endothelial aggressors might favor the clinical expression of low susceptibility genetic abnormalities (Figure 2). In other words, a genetic factor might confer a low risk of aHUS for individuals with intact kidneys but an increased risk of aHUS for renal transplant recipients who are sensitized by early endothelial injury. The crucial contribution of environmental factors in aHUS associated with dysregulation of the complement system is further supported by the contrast between the late-onset of aHUS observed in untransplanted individuals (occurring as late as the fifth decade of life in some cases) and the early-onset of aHUS (occurring within weeks) after transplantation.

Indications and scope of investigations

Our recommendations for the assessment of aHUS before transplantation differ only slightly from those formulated in the past couple of years.[48,49,94] All patients with aHUS should be screened for abnormalities in complement regulatory proteins as well as in ADAMTS13 activity before inclusion on renal transplant waiting lists. Of note, however, the distinction between typical HUS and aHUS, based on prodromic diarrhea, is not absolute.

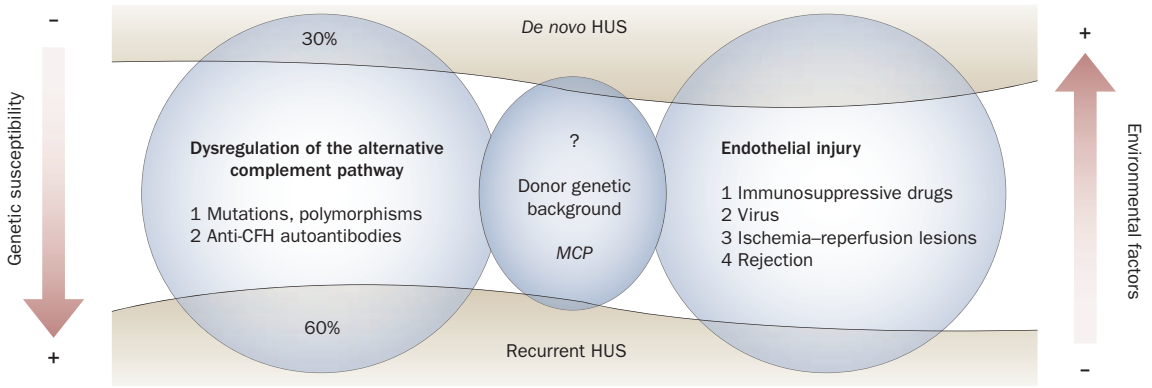

Figure 2 | Paradigm for post-transplant HUS. Post-transplant HUS can result from a complex interplay between donor and recipient genetic backgrounds as well as environmental factors that may trigger endothelial injury. Genetic abnormalities associated with complement dysregulation are identified in 30% and 60% of patients with post-transplant *de novo* HUS and recurrent HUS, respectively. This finding suggests that the contribution of genetic susceptibility to pathogenesis is lower in patients with *de novo* HUS than in those with recurrent atypical HUS (aHUS). It is likely, however, that the contribution of environmental factors has a greater role in the pathogenesis of post-transplant *de novo* HUS. In line with this assumption, *de novo* HUS has been linked to calcineurin-inhibitor-based immunosuppressive regimens, although the impact of calcineurin inhibitor on aHUS recurrence remains controversial. We propose that the presence of multiple endothelial aggressors in the post-transplant setting may reveal low-penetrance genetic susceptibility to HUS. Functional polymorphisms in *MCP* have been associated with the development and severity of aHUS. We therefore assume that the *MCP* genotype of the donor may influence the rate and severity of aHUS recurrence, although this hypothesis remains somewhat speculative. Abbreviations: CFH, complement factor H; HUS, hemolytic uremic syndrome; MCP, membrane cofactor protein.

Approximately 20–30% of cases of aHUS are preceded by diarrhea.[10,40] Furthermore, a genuine infection with Shiga-toxin-producing *E. coli* may initiate HUS, the evolution of which could be worsened by abnormalities in complement proteins.[10,19,95] Therefore, it seems reasonable to recommend investigations of complement components even in the context of a diarrhea prodrome, in the absence of Shiga toxin in the patient's stool and/or if the evolution was recurrent.

Differential diagnoses must be taken into account when considering the investigations that should be undertaken if a patient experiences *de novo* post-transplant HUS and/or subclinical TMA (Figure 3). Patients with prodromic diarrhea should be first screened for Shiga toxin. If Shiga-toxin-mediated disease is eliminated, the clinical context and associated histological lesions can provide conclusive arguments for a toxic, immune or infectious cause (Figure 3). If the cause of *de novo* post-transplant HUS cannot be determined or if the evolution of disease is unusually recurrent or chronic, despite resolution of the trigger event, the next diagnostic step should include a test for ADAMTS13 activity and investigations of the complement regulatory system.[33] Recommended investigations are described in Box 2. A list of laboratories that conduct these investigations is provided elsewhere.[3,49,96]

Importantly, normal levels of C3, CFH, CFI and CFB do not rule out genetic abnormalities. For instance, decreased levels of C3 were only found in 30% of aHUS patients with mutations in the complement regulators.[10,40] We believe, however, that protein levels of C3, CFH, CFI and CFB must nevertheless be measured. Although a complete genetic work-up usually requires several weeks, protein levels can be determined by immunohistochemical analysis within a couple of days. In addition, protein levels may provide important information about the functional significance of the identified mutation, which would help to discriminate between qualitative and quantitative defects. These findings may influence the assessment of prognosis and therapeutic management.

Specific issues of transplant settings

Donor–recipient genotype interactions

The donor genome might markedly influence outcomes after transplantation; however, the effect of donor genotype on aHUS recurrence after renal transplantation has not been studied. Theoretically, the donor genotype should control the expression of membrane regulatory proteins, such as MCP, complement decay-accelerating factor (DAF, also known as CD55), CD59, and thrombomodulin, which may be modified by functional genetic polymorphisms.[19,23,29,37,97,98] To date, only polymorphisms in *MCP* have been associated with aHUS, and it is likely that donor *MCP* polymorphisms could influence the risk of recurrence (Figure 2).[31] In addition, local secretion of C3 by the graft has been experimentally implicated in the pathogenesis of ischemia–reperfusion injury[61] and acute rejection.[60] In renal transplantation, donor C3 allotype has been correlated with functional prognosis,[99] although this finding remains controversial.[100] Nevertheless, the impact of donor C3 allotype and polymorphisms in complement components on aHUS recurrence after transplantation may be important to evaluate.

Risk of acute rejection

Observations from studies in experimental models suggest that the complement components C3 and C5

and the complement regulator DAF are involved in the enhancement and modulation of adaptive alloimmune responses, respectively.[60,101–104] In clinical transplantation, a few studies have shown that the rate of acute rejection in patients experiencing post-transplant HUS is increased in those with complement dysregulation.[40,45,105] However, calcineurin inhibitor withdrawal might be an obvious confounding factor in these studies.[105] Whether complement dysregulation alone augments susceptibility to acute rejection, independent of calcineurin-inhibitor-based immunosuppressive strategies, is currently impossible to determine.

Living donation in aHUS patients

Living-donor renal transplantation is still contraindicated in patients with aHUS associated with mutations in *CFH*, *CFI*, *C3* and *CFB*, given the unacceptable risk of disease recurrence,[48] although this recommendation will be reassessed in light of results from eculizumab trials. In aHUS patients with mutations in *MCP*, the physician should ensure that the donor does not share the same *MCP* mutation and rigorously exclude other genetic susceptibility factors before considering living-related donation. In addition, nephrectomy-induced rheologic changes can trigger aHUS in living-related donors who carry an unrecognized genetic susceptibility factor.[48] This occurrence has been reported four times in the literature.[48] Given the numerous unknowns regarding the clinical relevance of several polymorphisms and the increasing evidence for a polygenic pattern of aHUS disease, our opinion is that every case of living-related donation should be considered with caution. Indeed, one could never ensure that the mutation identified in the patient, and not in the living-related donor, is the only genetic susceptibility factor for HUS within the family.

Therapeutics: aims and tools

Avoiding drugs with endothelial toxicity

As discussed earlier, prolonged calcineurin-inhibitor-related ischemia is believed to induce endothelial damage. The benefit of calcineurin-inhibitor-free protocols in patients with post-transplant HUS remains controversial. Some studies have suggested that avoidance of calcineurin inhibitors might be beneficial in both recurrent and *de novo* forms of post-transplant HUS,[53,70,105] but others have reported conflicting results.[4,40,106,107] Various hypotheses could be proposed to explain these discrepant findings; however, the number of patients on calcineurin-inhibitor-free treatment in most studies may be insufficient for firm conclusions to be drawn.[4] Moreover, the most frequently used calcineurin-inhibitor-free protocol relied on a sirolimus-based regimen,[40] which is toxic to the endothelium.[88] In one study in which calcineurin inhibitors were withdrawn, most patients recovered from *de novo* post-transplant HUS after plasma exchange therapy. Interestingly, the majority of patients who were subsequently reintroduced to calcineurin inhibitors did not experience TMA recurrence. This finding suggests that calcineurin inhibitors can be safely reintroduced once TMA has been controlled with plasma exchange therapy and/or other aggressors of the endothelium, such as ischemia–reperfusion injury or rejection, has been treated or eliminated.[70] Finally, a maintenance immunosuppressive therapy based on belatacept, instead of a calcineurin inhibitor, has shown promising results in the prevention of post-transplant HUS.[108,109]

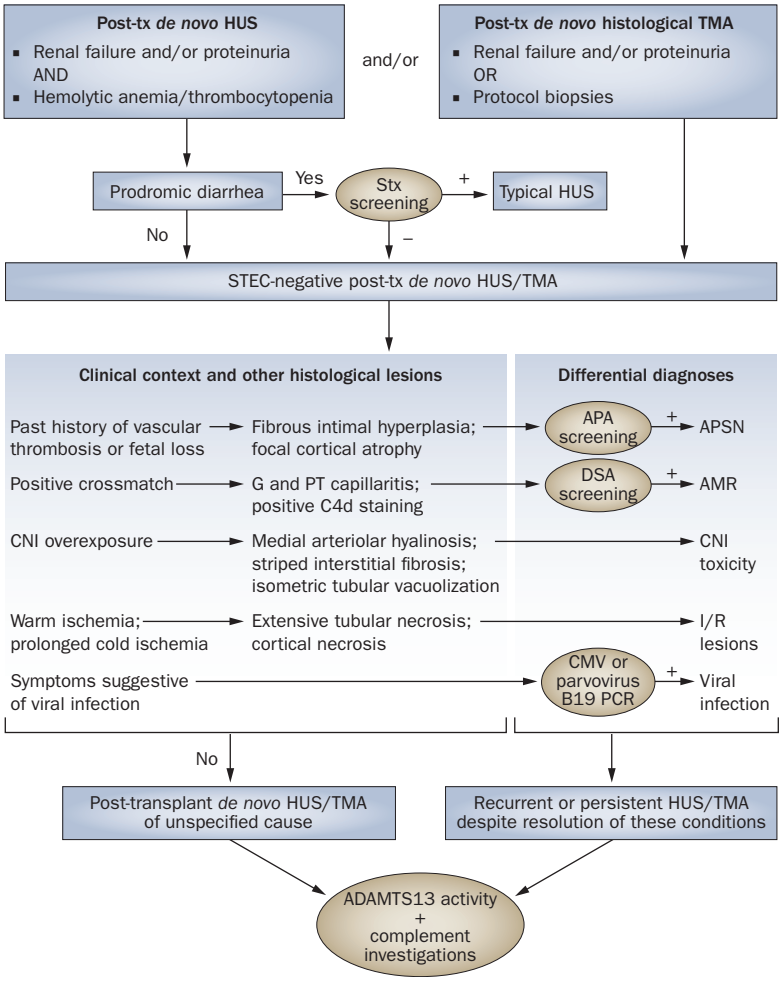

Figure 3 | Flowchart of investigations that should be undertaken if a patient experiences *de novo* post-transplant HUS and/or subclinical TMA. Abbreviations: AMR, antibody-mediated rejection; APA, antiphospholipid antibody; APSN, antiphospholipid syndrome nephropathy; CMV, cytomegalovirus; CNI, calcineurin inhibitor; DSA, donor-specific antibody; G, glomerular; HUS, hemolytic uremic syndrome; I/R, ischemia–reperfusion; PT, peritubular; STEC, Shiga-toxin-producing *Escherichia coli*; Stx, Shiga toxin; TMA, thrombotic microangiopathy; tx, transplant.

Pre-emptive and curative plasma exchange therapy

Plasma exchange therapy is the cornerstone of effective treatment for *de novo* post-transplant aHUS. Indeed, plasma exchange therapy, associated with calcineurin inhibitor withdrawal, achieved durable remission in 80% of patients with *de novo* post-transplant HUS.[70] By contrast, however, most studies have shown that plasma exchange therapy fails to prevent graft loss in patients with recurrent post-transplant HUS.[33,40,106,107] Therefore, use of pre-emptive plasma therapy has been proposed for patients with aHUS-related ESRD. Under this proposal, plasma exchange should be initiated just

Box 2 | Investigations of the complement system in aHUS

The following investigations are recommended.

Levels of complement factors*

- C3 (660–1,250 mg/l; nephelometry)
- CFH (338–682 mg/l; ELISA)
- CFI (42–78 mg/l; ELISA)
- CFB (90–320 mg/l; nephelometry)

MCP expression on peripheral blood leukocytes

Expression of MCP on peripheral blood leukocytes can be assessed by fluorescence-activated cell sorter analysis, and quantified by measuring the mean fluorescence intensity after staining with an anti-MCP antibody. Normal values depend on the antibody used.

Genotyping‡

- *CFH*
- *CFI*
- *MCP*
- *CFB*
- *C3*
- *THBD*

Screening for anti-CFH antibodies§

*Range of normal levels and technique used to measure levels are indicated in brackets. ‡By sequencing and multiplex ligation-dependent probe amplification. §By ELISA. Abbreviations: aHUS, atypical hemolytic uremic syndrome; C3, complement C3; CFB/H/I, complement factor B/H/I; ELISA, enzyme-linked immunosorbent assay; MCP, membrane cofactor protein; THBD, thrombomodulin.

before transplantation, continued as a daily treatment and then progressively tapered according to the evolution of disease.[32,40,49,110–113] To our knowledge, use of a pre-emptive strategy has been successful in preventing recurrent aHUS in eight renal transplant recipients, including patients with mutations in *CFH* ($n = 4$),[40,110–112] *CFI* ($n = 3$),[32,40] and *C3* ($n = 1$; J. Zuber, unpublished work) (Table 2). However, delayed recurrence may occur when plasma therapy is tapered.[40,111,112]

Attempts to reduce titers of anti-CFH antibody with pre-emptive plasmapheresis and/or rituximab has enabled successful renal transplantation in two patients with aHUS-related ESRD.[51,52] Cyclophosphamide therapy may also efficiently reduce anti-CFH antibody titer.[114] However, it is worthwhile stressing that this strategy achieves a metastable state (that is, the long-lived, but not interminable remission of aHUS), which would not preclude delayed recurrence of disease[75,111] after either a reduction in plasma support[111] or an infection.[75,111] Of note, increasing the plasma exchange frequency to daily sessions might restore baseline renal function in cases of recurrence despite pre-emptive plasma exchange, if it is done sufficiently early.[75,111] Prolonged anti-CMV prophylaxis might be useful in limiting the risk of CMV infection.[75,111]

Combined liver–kidney transplantation

A consensus conference held in Bergamo, Italy, in December 2007 led to the publication of recommendations for the use of combined liver–kidney transplantation for the treatment of aHUS.[49] The rationale for combined transplantation is based on the crucial role of the liver in the synthesis of circulating complement components, including CFH, CFI, CFB and C3. However, despite evidence from clinical proof-of-principal studies,[115,116] the first two reports of combined liver–kidney transplantation in carriers of *CFH* mutations were complicated by irreversible liver failure, associated with an extensive thrombotic microangiopathic process.[49,116,117] These initial reports suggested that surgical stress on the liver may induce the uncontrolled activation of complement in situations of complement dysregulation. Since the publication of these initial reports, a modified protocol involving perioperative anticoagulation and plasma exchange therapy was successfully used in seven of eight carriers of *CFH* mutations;[49,118–120] however, the remaining patient developed hepatic artery thrombosis after surgery and died of hepatic encephalopathy.[49] This fatal outcome is a reminder that combined liver–kidney transplantation is a high-risk procedure for which the benefit-to-risk ratio must be carefully evaluated. Auxiliary liver transplantation is not recommended in patients with aHUS because it may delay the diagnosis of acute rejection and create competition between mutant and non-mutant complement factors.[49] Patients who are eligible for combined liver–kidney transplantation include those with aHUS with mutations in *CFH* and *CFI*, especially those with a familial or personal history of disease recurrence after isolated kidney transplantation. Whether combined liver–kidney transplantation is appropriate for aHUS patients with mutations in *CFB* and *C3* remains unknown, owing to a lack of available information.

Eculizumab

Eculizumab is a monoclonal antibody that is specific for complement C5. It inhibits generation of the pro-inflammatory mediator C5a and the formation of the membrane attack complex (MAC), which is involved in the final effector pathway of complement activation. Eculizumab is currently approved for the treatment of paroxysmal nocturnal hemoglobinuria[121] and initial experience with eculizumab in patients with aHUS involving either native[122] or transplanted kidneys (Table 3)[110,113,123–125] have been extremely encouraging. Eculizumab treatment enabled withdrawal of plasma therapy,[110,113] led to the control of plasma-refractory forms of disease,[122–124] and was used prophylactically to prevent post-transplant recurrence of HUS.[125]

Of interest, although most patients in the above studies received eculizumab as a maintenance therapy (1,200 mg infusion every 2 weeks), two patients received only a single dose for the treatment of recurrent post-transplant HUS (Table 3).[123,124] Although both patients who received a single dose experienced a sustained response with a prolonged HUS-free period, delayed recurrence occurred in one patient after 11.5 months (M. Lozano, unpublished work),[124] and was presumed in the other after 21 months (J. Nürnberger, unpublished work).[123] The reintroduction of eculizumab led to remission of HUS in the former patient but failed to prevent

Table 2 | Studies of successful renal transplantation after pre-emptive plasma therapy

Study	Protein	Mutation or deletion	Recurrence on previous graft	Perioperative plasma therapy regimen	Maintenance plasma therapy	Duration of pre-emptive plasma therapy	Recurrence (context)	Response to PE following recurrence	Follow-up
Davin *et al.* (2010)[110]	CFH	Ser1191Leu	Yes (twice)	Daily PE over 1 week	PE twice per week	10 months (until the switch to anti-C5 Ab)	Yes (tapering of PE at 4 months PT)	Yes	16 months
Davin *et al.* (2008)[111]	CFH	Ser1191Leu	No	Daily PE over 1 week	Weekly PE	5 years	Yes (CMV infection)	Yes	5 years
Hirt-Minkowski *et al.* (2009)[112]	CFH	Arg1210Cys	No	Daily PI over 1 week	PI withdrawal	2 months	No	NA	12 months
Jablonski *et al.* (2009)[40]*; M. Essig, PC	CFH	Gly218Glu	No	Daily PI over 1 week	Monthly PI	26 months	No	NA	26 months
Cruzado *et al.* (2009)[32]	CFI; MCP	p.Cys247Gly; Cys210Phe	No	Daily PI over 10 days	PI and PE withdrawal	3 weeks	No	NA	9 months
Jablonski *et al.* (2009)[40]*; B. Moulin, PC	CFI	c.784delA; p.Gly243fsX46	Yes	PI at day 0 and day 1	PI every 2 weeks	2 years	Yes (at 2 years)	Incomplete response to PE (will be switched to anti-C5 Ab)	2 years
Jablonski *et al.* (2009)[40]*; E. Rondeau, PC	CFI	p.Arg474X	No	Daily PE over 5 days, followed by daily, progressively tapered PI	PI every 3 weeks	14 months	Yes (AMR at 12 months)	Yes	14 months
J. Zuber, unpublished work	C3	p.Lys155Gln	Yes	Daily PI	PI	3 years	No	NA	3 years
Le Quintrec *et al.* (2009)[52]	Anti-CFH	*CFHR1–CFHR3* del	Yes	Daily PI (14 days) followed by weekly PE	PE every 5 weeks	4.5 years	No	NA	4.5 years
Kwon *et al.* (2008)[51]	Anti-CFH	*CFHR1–CFHR3* del	No	Daily PE over 7 days	Progressively tapered	4 months	No	NA	2 years

*Outcomes have been updated since the manuscript was published with new unpublished data provided by the authors. Abbreviations: Ab, antibody; AMR, antibody-mediated rejection; C3, complement C3; CFH/I, complement factor H/I; CFHR, CFH-related protein; CMV, cytomegalovirus; MCP, membrane cofactor protein; NA, not applicable; PC, personal communication; PE, plasma exchange; PI, plasma infusion; PT, post-transplant.

graft loss in the latter. In another case report, a delay of 6 days in the ninth infusion of eculizumab led to a mild relapse of post-transplant aHUS, but the disease responded rapidly to the re-initiation of treatment.[113] Taken together, these data suggest that prolonged eculizumab therapy is more effective than a single dose, and that the 14-day delay between two doses should not be increased further. In addition to these off-label studies, which referred exclusively to case reports, four phase II multicenter and international trials have enrolled a total of 35 adult and adolescent patients with aHUS to establish the efficacy and safety of eculizumab.[126] Preliminary results from this trial should be communicated in the near future.

Drugs under development

Concentrated CFH, purified from human plasma, may be a future therapeutic option for carriers of *CFH* mutations, especially in those with a quantitative deficiency in functional CFH.[127] However, a number of unknowns exist with regard to the use of plasma-derived CFH therapy in patients with dominant–negative *CFH* mutations associated with dysfunctional protein. For instance, questions remain regarding its therapeutic efficacy, bioavailability, potential dosing, and complications relating to competition between the plasma-derived CFH and the mutated protein.

Recent data supporting a role for thrombomodulin in complement regulation suggests that this molecule may have therapeutic potential.[23] Use of a recombinant human thrombomodulin, which has anticoagulant and anti-inflammatory properties, was approved in Japan in 2008 for the treatment of disseminated intravascular coagulation (ART-123®). Of note, a 2009 publication reported the case of a patient who developed refractory TMA after hematopoietic stem cell transplantation; the TMA was successfully treated with recombinant thrombomodulin.[128] Other complement-modulating agents under development might represent additional therapeutic avenues.[129]

Table 3 | Post-transplant use of eculizumab for recurrent aHUS

Study	Protein	Mutation	Indication*	Period of disease recurrence before eculizumab initiation	Sustained period free of HUS	Therapeutic scheme	Eculizumab duration	Recurrence after eculizumab (delay after the last dose)	Follow-up (months)‡
Zimmerhackl *et al.* (2010)[125]; Jungraithmayr *et al.* (2009)[132]	CFH	Trp1183Cys	Primary prophylaxis	NA	Yes	1 dose at day 10, then 1 dose every 2 weeks	12 months	No	12
Davin *et al.* (2009)[110]	CFH	Ser1191Leu	Secondary prophylaxis	10 months	Yes	Complete protocol§	6 months	No	6
Chatelet *et al.* (2009);[113] B. Hurault de Ligny PC	C3	p.Arg592Gln	Secondary prophylaxis	14 months	Yes	Complete protocol§	24 months	Yes (3 weeks)	24
Nürnberger *et al.* (2009);[123] J. Nürnberger PC	CFH	Tyr475Ser	Curative	5 days	Yes	1 single dose (600 mg)	1 day	Likely but not biopsy proven (21 months)	22
J. Zuber, unpublished work	CFH	Ser1191Leu; Val1197Ala	Curative	4 days	Yes	Complete protocol§	6.5 months	No	6.5
Larrea *et al.* (2010)[124]; M. Lozano PC	NI‖	NI‖	Curative	9 days	Yes	1 single dose (600 mg)	1 day	Yes (11.5 months)	13

*Indicates whether eculizumab was used as a primary prophylaxis (to prevent the development of HUS recurrence), secondary prophylaxis (to prevent a second recurrence once the previous episode had been controlled by plasma therapy) or curative therapy (to rescue partial or complete plasma therapy failure). ‡Time of follow-up after initiation of eculizumab. §Administration of four doses of 900 mg eculizumab every 7 days plus a fifth dose of 1,200 mg 7 days after the fourth one, followed by a maintenance dose of 1,200 mg every 14 days. ‖Mutation screening included complete sequencing of *CFH*, *CFI* and *MCP* genes; a homozygous CFH_{TGTGGT} haplotype, also known as *CFH*–H3, was the sole genetic susceptibility factor for HUS identified in this patient. Abbreviations: aHUS, atypical hemolytic uremic syndrome; C3, complement C3; CFH, complement factor H; HUS, hemolytic uremic syndrome; NA, not applicable; NI, not identified; PC, personal communication (outcomes have been updated since the manuscript was published with new data provided by the authors).

Conclusions

aHUS, related to mutations in genes that encode circulating regulators of the complement system, has a poor prognosis and a high rate of recurrence after renal transplantation. Mutations responsible for cell-surface dysregulation of the alternative complement pathway have been identified in at least 60% of patients with aHUS. Therefore, biological and genetic study of the alternative complement pathway is a prerequisite for listing aHUS patients as candidates for renal transplantation. Emerging therapies, including pre-emptive plasma exchange therapy, combined liver–kidney transplantation, and eculizumab treatment, have increased the feasibility of such risky transplantations by efficiently preventing or treating disease recurrence. The finding that genetic complement abnormalities occur in approximately 30% of patients who develop *de novo* HUS after transplantation underscores the observation that genetic abnormalities in complement components can have a low penetrance and may be apparent only in favorable post-transplant environmental settings.

Review criteria

PubMed was searched for articles published up to May 31, 2010 using the following terms: “atypical hemolytic uremic syndrome”, “post-transplant HUS”, “alternative pathway of the complement system”, “eculizumab”, and “pre-emptive plasma therapy”. The reference lists of publications were also reviewed to identify additional relevant articles. We focused primarily on English-language, full-text papers.

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