Advances in understanding the aetiology of atypical Haemolytic Uraemic Syndrome

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Summary

Atypical Haemolytic Uraemic Syndrome (aHUS) is a thrombotic microangiopathy that often provokes irreversible renal damage and post-transplantation recurrence. Studies performed during the last decade have shown that 50–60% of aHUS patients present genetic or acquired defects in the complement system that enhance the initial endothelial damage and favour disease development. This review analyses the complement proteins and processes that are disturbed in aHUS patients, and outlines the relevance of a prompt genetic/molecular diagnosis for improving clinical management and prognosis.

Keywords: Haemolytic Uraemic Syndrome, complement system, anti-factor H autoantibodies, plasma therapy, transplantation.

Haemolytic Uraemic Syndrome (HUS) is clinically characterized by the simultaneous manifestation of haemolytic microangiopathic anaemia, thrombocytopenia and acute renal failure. Extra-renal involvement, mainly neurological, hepatic or digestive, can appear in up to 20% of patients. The incidence of HUS in children and adolescents is 5 times higher than in adults (Noris & Remuzzi, 2009). Classically, HUS has been categorized as typical and atypical. The typical form, also referred to as D+(diarrhoea positive)-HUS and accounting for 90–95% of cases in children, is characterized by abrupt onset following persistent diarrhoea in the preceding 2 weeks, and usually requires only supportive treatment. The prognosis is generally favourable, and only 5% of the patients die or evolve to end-stage renal disease (ESRD) in the short-term. In patients undergoing renal transplantation, recurrence is very rare. Atypical HUS (aHUS) represents 5–10% of paediatric cases of HUS, and the majority of adult cases. Diarrhoeal prodromes are less frequent and there may be an insidious clinical onset. aHUS has a poor prognosis, with a mortality or ESRD rate of 25% in acute episodes. The long-term outcome is also unfavourable: about 50% of patients who recover from the first episode evolve to ESRD, and disease recurrence after renal transplantation is high.

The anatomopathological findings observed in HUS correspond to thrombotic microangiopathy (TMA), with fibrin and platelet thrombi deposited in small arteries and capillaries of the kidney. TMA lesions are also observed in Thrombotic Thrombocytopenic Purpura (TTP) and in the HELLP (Haemolytic anaemia, Elevated Liver enzymes and Low Platelets) syndrome (Fang et al., 2008). Discrimination between HUS and these other entities has been mostly based on clinical criteria: the main organ affected in HUS is the kidney, in TTP neurological signs are predominant, and HELLP syndrome, which develops in pregnant women, is associated with liver involvement. Nonetheless, some HUS patients also present relevant neurological symptoms, and others develop the disease during pregnancy, thus complicating clinical diagnosis.

Most cases of typical HUS are associated with bacterial infections with the Escherichia coli strain O157:H7, Shigella dysenteriae or S. pneumoniae, secreting toxins or other molecules that provoke significant endothelial damage. The atypical forms were always considered to have an idiopathic origin. Genetic and immunological studies performed during the last decade have revealed that about 50% of aHUS patients present mutations in proteins of the complement system, a major component of innate immunity. Nonetheless, in many aHUS patients an infectious process (e.g. a viral infection, a vaccine or a diarrhoeal prodrome) is identified 1–2 weeks before HUS onset. On the other hand, a genetic component may also contribute to some typical HUS cases. Therefore, it appears that HUS is a multifactorial disease determined by environmental factors that damage the endothelium and initiate the pathogenic events, and by genetic factors that favour disease progression. In typical HUS environmental factors (e.g. bacterial toxins) are most relevant, while in the atypical forms there is a greater contribution of genetic factors. In this article we review the complement alterations associated with the atypical form of HUS, as well as the relevance of the complement findings to clinical management of patients.

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**Complement activation and regulation in aHUS**

The complement system is involved in the innate and adaptive immune response against infections, in the elimination of immune complexes and in the destruction and removal of autologous damaged cells. It is composed of a vast and heterogenous group of plasma proteins that are continuously circulating in the bloodstream in a ‘stand-by’ state, which become activated by specific molecules, such as antibodies or immune complexes (the classical pathway of complement activation) or by certain carbohydrate molecules present on the surface of several pathogens (the lectin pathway). In addition, there is a permanent, low-grade but spontaneous activation of complement that is amplified by specific pathogens, called the alternative pathway (Walport, 2001).

Complement activation initiates a series of protein interactions and enzymatic processes that generate different kinds of effector molecules, which in turn will allow direct destruction of the pathogen by osmotic lysis, or by enhancing phagocytosis or inflammation. This sequential mechanism is potentially dangerous for the host: excessive activation will consume all the effector molecules, increasing susceptibility to infections. On the other hand, the effector molecules can accumulate on nearby host cells and tissues, inducing damage. Fortunately, there is a group of complement components which perform a regulatory function on the whole system. Some of these complement regulators circulate in plasma and others are expressed on the surface of most cells, so that in a normal situation all host tissues are protected against damage by autologous complement.

It is precisely this equilibrium between complement activation and regulation that is disturbed in aHUS patients presenting complement defects. All complement proteins that have been found mutated in these patients participate in the central, and probably most critical, process for a proper complement response: the generation and further inactivation of the C3b molecule through the alternative pathway (Fig 1). Small amounts of C3b are continuously being generated in plasma and on the surface of host cells, but this process is limited by several regulatory mechanisms, and the most important is the proteolytic digestion of C3b into iC3b. The generation of iC3b abruptly turns off the complement cascade, avoiding complement consumption and damage to autologous cells and tissues.

In aHUS patients presenting with mutations in complement proteins, there is an excessive generation of C3b and/or a defective inactivation of this molecule into iC3b. As a consequence, in a situation of endothelial cell damage that induces complement activation (e.g. an infection), large amounts of C3b molecules will be deposited on the endothelial cell surface and the complement cascade will proceed into cell damage or destruction by the cytolysic Membrane Attack Complex. Amplification of the initial endothelial damage will then favour prothrombotic processes and HUS development.

**Overview of complement defects in aHUS patients**

Studies on complement genes and proteins have mostly been performed in five cohorts of aHUS patients, from Italy, France, Germany, United Kingdom and Spain; an American aHUS cohort has been recently described (Maga et al, 2010). Genetic defects include mutations only reported in aHUS patients, common polymorphisms that predispose to the disease, or a combination of both types of alterations. The only acquired complement defect in aHUS patients thus far described is the presence of circulating anti-factor H autoantibodies, although even this seems to be associated with genetics, as discussed in section Defects in the regulators of the alternative pathway. The most relevant complement findings in aHUS patients are summarized in Table I.

**Type of genetic defects**

*Mutations.* Mutations in Complement components have been reported in 40–60% of aHUS patients. In most, the mutation is heterozygous, but some patients are homozygous for a mutation, and others are compound heterozygotes (each allele presents a different mutation). Some mutations provoke defects that result in reduced levels of the mutated proteins (type I mutations), while others provoke defects in the activity of the mutated protein, but not in the protein level (type II mutations). An updated list of reported mutations can be found at the factor H (FH)-HUS mutation website (http://www.fh-HUS.org; Saunders et al, 2007).

Five complement genes have been found to be mutated in aHUS patients: factor H (CFH), CD46 (CD46), also known as Membrane Co-factor Protein, factor I (CFI), factor B (CBF) and C3 (C3). The most frequent genetic defects found in the patients are single base mutations, followed by deletions or insertions of a few consecutive nucleotides. These kinds of mutations have been found in the five genes. Other mutations identified in aHUS patients are consequences of the high sequence similarity between factor H and the CFHR (Complement factor H-Related) protein family (Józsi & Zipfel, 2008). The CFH gene and the five CFHR genes (CFHR1–5) are located in close proximity on chromosome 1q32, and share several duplicated regions that favour non-homologous recombination processes between them. Thus, a hybrid CFH-CFHR1 gene, in which the last 2 exons of CFH have been substituted for the last 2 exons of CFHR1, has been reported (Venables et al, 2006); this hybrid gene originates a mutant factor H protein with 2 aminoacid changes in its C-terminal domain. Other factor H mutants are generated by gene conversion processes between the CFH and CFHR1 genes (Heinen et al, 2006).

*Risk polymorphisms/haplotypes.* The five complement genes that are mutated in aHUS patients (CFH, CD46, CFI, CBF and C3) are polymorphic, i.e. they present different variants in the
normal population. Interestingly, some of these polymorphisms are more frequent in aHUS patients than in unaffected individuals. For this reason, they are referred to as ‘risk polymorphisms’ or ‘risk haplotypes’ (a combination of polymorphisms). Although the functional relevance of these polymorphisms for HUS pathogenesis has not been established, family studies in aHUS patients reveal that affected members often present a risk polymorphism in addition to the mutation, suggesting that polymorphisms modulate disease penetrance.

A polymorphism in C4b-binding protein, a regulator of the classical pathway of complement activation, has been associated with aHUS in the French and United Kingdom cohorts (Blom et al., 2008), but not in the Spanish cohort (Martinez-Barricarte et al., 2008). A recently described polymorphism in the CFHR1 gene is associated with aHUS in the Spanish cohort of patients (Abarrategui-Garrido et al., 2009), but whether this is also true in the other cohorts remains to be determined.

**Combined mutations.** Some aHUS patients present mutations in two or three different complement genes. This fact was studied in a family where the two affected members presented three genetic predisposing factors: a mutation in CD46, a mutation in factor I and a risk haplotype in CD46 (Esparza-Gordillo et al., 2006). Other CD46 combined mutations are CD46+ CFH (Caprioli et al., 2006), and CD46+ CFH+ CFI (Bienaime et al., 2010). Recently, a patient presenting a mutation in CD46 and in the complement regulator...
clusterin has been described (Ståhl et al., 2009). These observations illustrate that the regulatory complement network is generally very efficient: some regulators perform redundant or overlapping functions, so that a defect in one single regulator can be compensated for by the normal function of the others. The existence of combined mutations also supports the multigenetic basis in aHUS pathogenesis, and illustrates the need to study all relevant complement genes in every aHUS patient.

Other genetic factors: thrombomodulin. The first mutations outside the complement system were recently identified in the thrombomodulin gene (THBD) (Delvaeye et al., 2009). THBD mutations were found in about 5% of the aHUS patients studied, none of whom presented mutations in the Complement genes. The authors show that thrombomodulin binds C3b and enhances its irreversible inactivation to iC3b, and conclude that the mutations found in aHUS patients reduce this complement regulatory function of thrombomodulin. These mutations, nonetheless, also increase thrombomodulin binding to C3b, an observation in apparent contradiction with reduced C3b inactivation. Therefore, whether the pathogenic mechanism of THBD mutations in aHUS directly involves the Complement system, or it is mostly related to thrombomodulin function in the coagulation/fibrinolytic pathways may require further investigation.

Acquired defects

Anti-factor H autoantibodies. Some aHUS patients who do not present mutations in the complement genes have circulating antibodies that recognize factor H. Most of these patients are children between 3 and 17 years of age and they present homozygous deficiency of CFHR1 and CFHR3. This observation suggests a genetic basis for the generation of factor H autoantibodies that is currently under investigation. Anti-factor H autoantibodies can also be found in patients who have mutations in complement genes, again illustrating that HUS onset often requires the concurrence of genetic and acquired predisposing factors.

Defects in the regulators of the alternative pathway

The most frequent complement defect in aHUS patients is defective regulation of the alternative pathway as a consequence of the reduced levels or the anomalous function of factor H, factor I or CD46.

Factor H

Factor H is a plasma glycoprotein that regulates complement activation in plasma and on cellular surfaces. It is organized by

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20 structural units named Short Consensus Repeats (SCRs), also known as Complement Control Protein (CCP) modules. Factor H deficiency allows systemic activation of the alternative pathway of the complement system, giving rise to low or very low plasma levels of C3. Mutations generating homozygous deficiency of factor H in aHUS patients are infrequent, while mutations generating heterozygous factor H deficiency have been reported in a higher number of patients (Dragon-Durey et al., 2004). However, most factor H mutations identified in aHUS patients are heterozygous missense mutations that do not provoke factor H deficiency. These mutations are located in the C-terminal domains of the molecule, especially in SCR20, within a factor H region that is critical for complement regulation on surfaces (Caprioli et al., 2001; Pérez-Caballero et al., 2001; Richards et al., 2001). Accordingly, some of these missense mutations have been shown to provoke defective binding of factor H to surface-bound C3b, without affecting its regulatory activity in the fluid phase (Sánchez-Corral et al., 2002), while a reduced binding of factor H to endothelial cells (Manuelian et al., 2003) or to platelets (Stähli et al., 2008) was observed in other mutations. Therefore, the functional defect of the mutated factor H mainly alters complement regulation on cellular surfaces, but not in plasma; this explains why most of the patients present normal levels of factor H and the central components C3 and C4. In line with these findings, transgenic mice expressing a truncated factor H that lacks the five C-terminal domains spontaneously developed HUS (Pickering et al., 2007), while the factor H knockout mice presented a phenotype of membranoproliferative glomerulonephritis (Pickering et al., 2002).

HUS penetrance in carriers of factor H mutations is about 50% (Caprioli et al., 2006), suggesting the existence of additional genetic susceptibility factors. Several single-nucleotide polymorphisms (SNPs) in CFH were initially associated with the disease (Caprioli et al., 2003), and further studies identified factor H haplotypes that confer a higher risk to HUS, as well as haplotypes that are protective (Pickering et al., 2007; Tortajada et al., 2009). Combined mutations of factor H with other complement proteins have also been reported (Caprioli et al., 2006; Bienaime et al., 2010; Maga et al., 2010).

**Factor H autoantibodies**

Circulating factor H autoantibodies were first described in three paediatric aHUS patients without mutations in factor H, factor I or CD46, and were proposed to reduce the regulatory activity of factor H on the cellular surface (Dragon-Durey et al., 2005). Some of these autoantibodies have been characterized and shown to recognize the SCR20 domain of factor H (Józsi et al., 2007). Functional studies reveal that the autoantibodies provoke the same dysfunction in factor H as the mutations in the SCR20 domain, and there seems to be a direct correlation between the extent of the factor H dysfunction and the amount of CFH: autoantibody complexes (Strobel et al., 2010).

Most patients with anti-factor H autoantibodies present homozygous deficiency of the factor H-related proteins CFHR1 and CFHR3 as a consequence of the homozygous deletion of the two genes (Zipfel et al., 2007; Józsi et al., 2008; Lee et al., 2009). These findings suggest a role for CFHR1 and/or CFHR3 in this autoimmune form of HUS, and a denomination of DEAP-HUS (DEFiciency of CFHR plasma proteins and Autoantibody Positive form of Haemolytic Uraemic Syndrome) has been proposed for this group of patients (Zipfel et al., 2010). Recent studies have shown that a few patients with autoantibodies present homozygous deficiency of CFHR1, but not of CFHR3 (Abarategui-Garrido et al., 2009; Moore et al., 2010). Interestingly, the CFHR1 deficiency in those patients result from different genetic alterations, such as the genomic deletion of CFHR1 and CFHR4, or the presence of mutations in CFHR1. Thus, it seems that it is CFHR1 (and not CFHR3) that has a role in DEAP-HUS. In this context, it is noteworthy that the factor H domain recognized by the autoantibodies is highly similar to the SCR5 domain of CFHR1, and that some anti-factor H autoantibodies crossreact with CFHR1 (Moore et al., 2010). Anti-factor H autoantibodies can present together with CFH, CFI or CD46 mutations (Moore et al., 2010).

**CD46**

The complement regulator CD46 (also known as Membrane Co-factor Protein, MCP) is expressed on the surface of most cellular types and tissues, including the kidneys. CD46 binds C3b through its extracellular domains, and acts as a cofactor of factor I in the proteolytic inactivation of C3b into iC3b.

CD46 mutations were first identified in familial cases of aHUS from the Italian and Newcastle cohorts and later on in nine aHUS French pedigrees (Niris et al., 2003; Richards et al., 2003; Frémeaux-Bacchi et al., 2006). Most CD46 mutations are located in the 4 extracellular SCR domains and result in reduced expression of the protein (Couzi et al., 2008). A few mutations present with normal CD46 expression and are thought to provoke a functional defect, such as reduced C3b binding (Richards et al., 2003). In most patients the mutation is heterozygotic, although homozygous mutations have also been reported (Richards et al., 2003; Frémeaux-Bacchi et al., 2006), and the majority present normal levels of C3 and C4 in plasma.

An CD46 haplotype integrated by 5 SNPs confers a higher risk to aHUS and modulates disease penetrance (Esparza-Gordillo et al., 2005; Frémeaux-Bacchi et al., 2005). This risk haplotype was present in affected members of a Spanish family with combined mutations in CD46 and factor I, thus illustrating the concurrence of several genetic predisposing factors in aHUS development (Esparza-Gordillo et al., 2006).
Factor I

Factor I is the complement enzyme that cleaves C3b and generates the inactive form iC3b. It is a serine protease that circulates in plasma in active form, but it requires the presence of factor H or CD46 acting as cofactors.

Factor I mutations thus far identified in aHUS patients are heterozygous and in most cases they associate with low levels of factor I and C3 (Frémeaux Bacchi et al, 2004; Kavanagh et al, 2005; Nilsson et al, 2010); homozygous deficiency of factor I has only been described in one patient (Bienaime et al, 2010). Mutations are frequently located in the serine protease domain of factor I, and some of them generate a dysfunctional protein that cannot properly cleave fluid-phase C3b or surface-bound C3b (Caprioli et al, 2006; Kavanagh et al, 2008; Bienaime et al, 2010; Nilsson et al, 2010).

Incomplete disease penetrance has also been shown for factor I mutations (Kavanagh et al, 2005). Thus far, no factor I risk polymorphisms have been reported, but a high incidence of combined mutations in this group of patients has been observed (Bienaime et al, 2010), suggesting that the complement defect provoked by a partial factor I deficiency is not as relevant for aHUS development as, for example, a dysfunctional factor H protein.

Defects in the activators of the alternative pathway

The mutations in the complement activating components factor B and C3 identified in aHUS patients are thought to enhance the generation and stability of the C3 convertases, thus increasing the amount of C3b molecules generated.

Factor B

Factor B provides the catalytic site for the C3-Convertases of the alternative pathway, but it circulates in plasma in an inactive form that becomes activated upon binding to C3b and further proteolytic cleavage by factor D (Fig 1). A total of four different factor B mutations have been described in aHUS patients from the Spanish and French cohorts (Goicoechea de Jorge et al, 2007; Roumenina et al, 2009).

The four mutations are located in the factor B domain which interacts with C3b, and they increase the rate of generation of the C3-convertase complex, or enhance its stability against complement regulators factor H or Decay Accelerating Factor. Thus, these are gain-of-function mutations that provoke excessive activation of the alternative pathway, and therefore associate with low C3 levels in the patients’ sera. It is noteworthy that two of the mutations are ‘de novo’ mutations, while the others correspond to familial cases of aHUS. In one of these families, 7 of the 11 mutation carriers were disease affected, and they also presented a homozygous CD46 risk haplotype (Goicoechea de Jorge et al, 2007). New factor B mutations have been recently described, but functional studies have not been performed (Maga et al, 2010; Tawadrous et al, 2010).

C3

C3 is the central component of the complement system, and it circulates in plasma at high concentrations. It is a substrate for the C3 convertases, and its active product C3b is also a component of these enzymatic complexes (Fig 1). Mutations in C3 have been described in 11 unrelated patients from two cohorts, all presenting persistently low levels of C3 (Frémeaux-Bacchi et al, 2008). Two mutations provoked C3 deficiency, while five mutations increased the resistance of the mutated C3b to irreversible inactivation by factor I and CD46, and are considered gain-of-function mutations. Familial studies in one of the patients revealed that three of the mutation carriers were healthy and others presented hypertension, haematuria or chronic kidney disease; of note, the only aHUS patient in the family presented the CD46 risk haplotype in addition to the C3 mutation (Lhotta et al, 2009).

Management

Studies performed in different cohorts of aHUS patients have revealed that the patient’s evolution and response to treatment is largely determined by the underlying complement defect (Table II). Many of these studies have been possible because of the availability of registries of aHUS patients, in which clinical, laboratory and genetic/molecular data have been incorporated (Neumann et al, 2003; Caprioli et al, 2006; Sellier-Leclerc et al, 2007). The majority of the reported data are from paediatric patients; for patients with an adult onset the available information in this context is very limited. Current patient registries are the International Registry of Recurrent and Familial HUS/TTP, the International Registry and Biorepository for TMA, and the Innsbruck registry for HUS.

Mutations in factor H are the most frequent (15–30%) and have the poorest prognosis, but patients may evolve satisfactorily upon plasma therapy. Mutations in CD46 present in 10–15% of patients and although they associate with multiple HUS episodes, renal function is generally recovered and HUS recurrence after transplantation is infrequent. Factor I mutations appear in 5–10% of patients and have a variable prognosis, generally unfavourable; the outcome in patients with combined mutations in factor I seems to be worse than in patients with single mutations. Patients presenting mutations in factor B also have a poor prognosis, although very few data are available. Approximately 50% of the reported patients with C3 mutations have a favourable outcome and do not present recurrences after renal transplantation. Therefore, aHUS patients must be diagnosed as soon as possible, and the molecular/genetic studies that could modify the initial treatment and determine future therapeutic options should be performed in parallel. A schematic protocol for the initial therapeutic and diagnostic approach is depicted in Fig 2.
A more detailed and exhaustive guideline focused on clinical practice has recently been published (Taylor et al, 2010).

**Initial treatment**

According to the general clinical experience, it seems that prompt initiation of plasma exchange (PE) treatment decreases mortality and the risk of ESRD during the first HUS episode. PE with fresh frozen plasma (FFP) must be initiated within 24 h of diagnosis, in parallel with standard supportive treatment for acute kidney damage. A protocol for PE treatment during the first month after HUS onset has been recently described (Ariceta et al, 2009). This protocol should be modified according to the clinical situation of the patient, and to the results of informative diagnostic parameters. As a rule, the amount of exchanged plasma should not be lower than 1–2 plasma volumes in adult patients, and 50–100 cc/kg in paediatric patients.

The rationale for PE as the treatment choice is that defects in complement proteins are the most frequent alterations in aHUS patients. Mutated proteins with an abnormal function (factor H, factor I, factor B, C3) and anti-factor H auto-antibodies will be removed by plasmapheresis, while the exogenous plasma will provide additional amounts of fully functional complement proteins. An additional advantage of PE over isolated plasma infusion is that higher amounts of exogenous plasma can be provided, with a lower risk of hypervolaemia, a complication frequently observed in patients with a defective renal function.

**Treatment modifications according to the complement findings**

About 50% of the aHUS patients will present mutations in complement components or anti-factor H autoantibodies. The molecular findings can help the clinician adjust the treatment in these patients. As genetic studies will require 1–2 months to be completed, the initial treatment during the aHUS episode must be carried out without any genetic information available. Nonetheless, many of the complement defects already found in aHUS patients can be detected with immunological assays that can be completed within 1–3 weeks from HUS onset, and provide a molecular basis for some therapeutic options that could improve the clinical course of the disease.

**Patients with anti-factor H autoantibodies.** The enzyme-linked immunosorbert assay (ELISA) for detecting anti-factor H auto-antibodies should be performed as soon as possible, especially in paediatric patients between 3 and 17 years. The plasmapheresis treatment in these patients should be enhanced

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<th>Complement defect</th>
<th>Frequency</th>
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ESRD, end-stage renal disease; PE, plasma exchange.

*Most of the series include only patients with paediatric onset, therefore the age at onset is the minimal age reported. Few data about adult patients are available.

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in order to eliminate or reduce circulating autoantibodies, and parallel immunosuppressive treatment would also be initiated. Immunosuppressants, such as corticosteroids, azathioprine, mycophenolate and rituximab have been used with variable results; recently, two cases were successfully treated with cyclophosphamide (Boyer et al., 2010). PE combined with corticosteroids and rituximab has been successful in the only adult patient with autoantibodies thus far reported (Lionet et al., 2009). It is important to note that plasmapheresis treatment for autoantibody removal will also eliminate factor H; therefore, replacement with plasma instead of with albumin solutions is particularly recommended.

The levels of pathogenic anti-factor H autoantibodies among aHUS patients are variable, and there is not clear relationship between the autoantibody titre and the clinical situation. However, for an individual patient, an increase in the autoantibody titre is generally associated with worse clinical symptoms and reduced C3 levels, most likely as a consequence of lower amounts of free factor H available. Autoantibodies can persist for many years after HUS onset, and monitoring of the autoantibody titre may help follow patients’ evolution and anticipate disease relapses (Le Quintrec et al., 2009).

### FIG 2. Protocol for the initial treatment and the molecular/genetic diagnosis

The clinical/treatment algorithm is based on Ariceta et al., 2009.

#### (1) If the molecular/genetic studies cannot be initiated immediately, the blood sample must be centrifuged and the EDTA-plasma stored at \(-20^\circ\text{C}\) for further analyses. HIV, human immunodeficiency virus; ANAs, anti-nuclear antibodies.

| Patients with mutations which provoke deficiency in factor I, factor H or MCP. About 50% of patients with mutations in factor I, and a few patients with mutations in factor H will present reduced levels of these proteins in plasma, and will

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<td>Fifth week</td>
<td>3 Sessions</td>
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- Enhance PE Immunosuppressant therapy
- Low CFH/CFI levels
- Low CD46 expression

**C3/C4 levels**
**CFH autoantibodies**
**CFH, CFI, CFB levels**
**CD46 expression**

**Others (according clinical suspicion):**
- HIV, ANAs, Anti-Phospholipid Ab, ADAMTS-13 activity

**DNA extraction for genetic studies:**
Not necessarily before treatment.

**5 ml Blood-EDTA for measuring:**
- C3/C4 levels
- CFH autoantibodies
- CFH, CFI, CFB levels
- CD46 expression

*Enhance PE Immunosuppressant therapy*

**Consider PE withdrawal**

**Gene analysis:***
3 sessions

**Genetic data (Available 1–2 months after onset):***
- DNA extraction for genetic studies
- Not necessarily before treatment

**Immunological data (Available 1–3 weeks after onset):***
- Low CFH/CFI levels
- Low CD46 expression
- Continue PE or initiate Plasma Infusion
thus be identified by immunological studies. In these patients there is no need to remove any altered protein, so it is possible to choose between PE or plasma infusion to provide additional factor I or factor H. In addition, a prophylactic strategy based on periodic plasma infusions or exchanges can be adopted; monitoring of factor I and factor H levels will facilitate decisions about doses and timing.

Most patients with mutations in CD46 will show a reduced expression of this protein on peripheral blood leucocytes and can be easily and promptly detected by flow cytometry. Because CD46 is a membrane protein, the plasma treatment will not provide additional CD46, and similar results are obtained with or without PE. Nonetheless, the decision to stop the plasma treatment in these patients should take into account that some can present mutations in other complement proteins (mainly factor I and factor H) that may be difficult to diagnose early.

**Patients with mutations which provoke dysfunctions in factor H, factor I or MCP.** Most patients with mutations in factor H will present normal factor H levels in plasma and they will not be identified with standard immunological techniques. A similar situation occurs in some patients with factor I mutations, and in a few patients with CD46 mutations. Early identification of these patients will require functional assays specific for each of these proteins.

The only functional assay thus far described is an ‘in vitro’ haemolytic assay that is performed with the patient’s serum and detects mutations that generate dysfunctional factor H molecules (Sánchez-Corral et al., 2004; Váziri-Sani et al., 2006). The assay can be easily performed in immunology laboratories. In our experience, it is very useful for the screening of missense mutations in SCRs 19–20 of factor H (75% sensitivity), and it also identifies patients with high titres of factor H autoantibodies. We have also used the assay to monitor the efficacy of plasma treatment in one aHUS patient (Abarrategui-Garrido et al., 2008).

Unfortunately, the majority of the patients with mutations that generate dysfunctional proteins will not be identified until the genetic studies are completed, and the protocol of PE in these patients will have to be modified based exclusively on individual clinical response.

**Patients with low levels of C3.** The levels of the central complement components C3 and C4 in plasma are easily determined by standard nephelometry. A few patients will present normal levels of C4 with low C3 levels, suggesting anomalous activation of the alternative pathway. If factor H and factor I levels in these patients are normal, and they do not present anti-factor H autoantibodies, a mutation in factor B or in C3 could be suspected, although the definitive diagnosis would be genetic. Plasma therapy was effective in three out of four patients with gain-of-function mutations in factor B (Goicoechea de Jorge et al., 2007; Roumenina et al., 2009). For patients with mutations in C3, it has been proposed that prompt and extensive PE treatment may greatly influence the recovery of renal function (Frémeaux-Bacchi et al., 2008; Lhotta et al., 2009). Monitoring of C3/C4 levels could help evaluate the efficacy of the treatment.

**Future treatments**

aHUS patients presenting complement mutations may benefit in the immediate future from complement-inhibiting therapies. A humanized monoclonal antibody against complement component C5, which is currently used for the treatment of paroxysmal nocturnal haemoglobinuria (Eculizumab), has been shown to be effective in a few aHUS patients (Chatelet et al., 2009; Gruppo & Rother, 2009; Mache et al., 2009; Nürnberg et al., 2009; Davin et al., 2010), and an international clinical trial is ongoing.

Another more specific therapeutic alternative for the future is the use of a factor H concentrate prepared from human plasma that was designated an ‘Orphan Drug’ by the European Medicines Agency in January 2007, and which is currently under pre-clinical development. This will be an appropriate choice for patients presenting factor H deficiency/dysfunction and it could also benefit patients with deficiencies in the other regulators.

**Transplantation**

The outcome of renal transplantation in aHUS patients is much worse than in typical cases, in particular because of the high incidence of disease recurrence, which often provokes failure in the transplanted kidney (Loirat & Frémeaux-Bacchi, 2008). Most data refer to patients with mutations in factor H, who show 80% of disease recurrence post-transplantation. A similar or even higher recurrence rate is expected for patients with mutations in factor B, although very limited data are available, while in the case of factor I recurrence is observed in 50–90% of patients. Renal transplantation in patients with C3 mutations may have a better prognosis, as suggested from the 40% of HUS recurrence observed for three different mutations (see Table II for references).

The situation in patients with mutations in CD46 is much better. As CD46 is a membrane protein expressed on most cellular types, the transplanted kidney will express a normal CD46 capable of appropriately regulating complement activation on its surface, and a low recurrence rate is expected. Post-transplant recurrence has been reported in a patient suspected to present endothelial microchimerism (Frémeaux-Bacchi et al., 2007).

Successful renal transplantation after intensive PE has been reported in two patients with anti-factor H autoantibodies. In one of these patients, rituximab was used in addition to PE to inhibit autoantibody production (Kwon et al., 2008). The other patient had failed four previous renal grafts, two by HUS recurrence; in the fifth transplant regimen, PE was added to immunosuppression, with no recurrences after 36 months of
follow-up (Le Quintrec et al., 2009). These experiences suggest that a high autoantibody titre is a risk for post-transplant HUS recurrence. Three patients from the Newcastle cohort were successfully transplanted without any specific pre-transplant therapy, but the autoantibody diagnosis in these patients was retrospective and it is possible that the autoantibody titre before transplantation was low (Moore et al., 2010; Waters et al., 2010).

The high rate of disease recurrence observed in patients with factor H mutations suggested that isolated liver or combined liver-kidney transplantation might be a better option. Isolated liver transplantation has only been reported in one patient with factor H deficiency; the factor H levels normalized but the child suffered from serious infectious complications and died 11 months after the transplant (Cheong et al., 2004). The first experiences of combined kidney-liver transplantation were unsuccessful because of immediate liver failure due to acute humoral rejection or to massive thrombotic microangiopathy (Remuzzi et al., 2002, 2005). Nonetheless, these first cases indicated that complement activation on the transplanted organs must be avoided, and a successful liver-kidney transplant has been reported (Saland et al., 2006). Intensive pre-operative plasmapheresis and plasma infusion during surgery were incorporated, as well as anticoagulation with low-molecular-weight heparin and aspirin. Standard immunosuppression (including calcineurin inhibitors) was used after transplantation. A total of four successful liver-kidney transplants have been reported (Jalanko et al., 2008; Saland et al., 2009a).

In December 2007 a consensus conference to evaluate the experiences in isolated or combined transplantation in aHUS patients was held in Bergamo. The transplant recommendations according to genetic findings are summarized in Table III. For patients with ‘gain-of-function’ mutations in factor B or C3 there is a theoretical risk of disease recurrence associated with extra-hepatic production of the mutated protein. A combined liver-kidney transplant in a patient with a mutation in factor B was performed in March 2009 in our hospital. The patient has not experienced any disease recurrence, and presents normal hepatic and renal functions. Experience with patients with combined mutations is very limited, but a successful kidney transplant has been reported in a patient with combined mutations in CD46 and factor I (Cruzado et al., 2009). Nonetheless, indications for liver or combined liver-kidney transplantation will probably change in upcoming years if the efficacy of Eculizumab is confirmed. In this context, a successful renal transplantation combined with Eculizumab has been recently reported in one patient with a mutation in factor H (Zimmerhackl et al., 2010).

Finally, living-donor transplantation in patients with aHUS is currently contraindicated because of the high risk of disease recurrence. In addition, if the donor is an asymptomatic mutation carrier he may develop the disease after the surgical stress (Donne et al., 2002). If the procedure is considered, donor and recipient must be genotyped for any

<table>
<thead>
<tr>
<th>Table III. General recommendations for transplantation in aHUS patients with complement defects.</th>
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<tr>
<td>Patients with mutations in factor H or factor I</td>
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<tr>
<td>Liver or combined liver-kidney transplantation</td>
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<tr>
<td>Consider isolated kidney transplantation if:</td>
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<tr>
<td>Mutations are associated with successful isolated kidney</td>
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<tr>
<td>transplantation in affected family members</td>
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<tr>
<td>Low-risk CFH or CFI mutation (i.e. mutations reported as not</td>
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<td>being associated with recurrence after isolated renal</td>
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<td>transplantation)</td>
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<td>Patients with MCP mutations</td>
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<td>Isolated kidney transplantation</td>
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<tr>
<td>Patients with mutations in factor B or C3</td>
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<tr>
<td>Liver or combined liver kidney transplantation (few experiences, but</td>
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<td>high recurrence rates after isolated kidney transplant)</td>
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<td>Patients with combined mutations</td>
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<tr>
<td>No sufficient information available.</td>
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<td>Patients with anti-factor H autoantibodies</td>
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<td>Isolated kidney transplantation when the autoantibody titre is low</td>
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</table>

Based on Saland et al., 2009b. Isolated and combined transplantation must be performed with intensive perioperatory plasma therapy, except in patients with isolated CD46 mutations.

disease-associated mutation (Noris & Remuzzi, 2009). Bilateral nephrectomy may be necessary pre-transplantation to control disease activity but it will not modify the recurrence rate (Saland et al., 2009b).

Conclusions

Defective functioning of the alternative pathway of the complement system is a major pathogenic mechanism in aHUS. Mutations in the complement regulators factor H, factor I or CD46, or in the complement activators factor B and C3, break down the normal activation-regulation balance, provoking complement damage to host cells and tissues. Additional complement genetic susceptibility factors may be associated with factor H-related proteins, particularly in the autoimmune form of aHUS. Concordance of several genetic/acquired susceptibility factors modulate disease penetrance. Most aHUS patients present a normal complement profile in plasma, and identification of the underlying complement defect requires that immunological and genetic studies be initiated as soon as possible. A prompt molecular diagnosis allows implementation of therapeutic strategies that may improve the patient’s outcome.

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