

# Hemolytic uremic syndrome recurrence after renal transplantation

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**Abstract:** About 60% of non-Stx-associated aHUS are due to the defect of protection of endothelial cells from complement activation, secondary to mutations in the genes of CFH, MCP, IF, BF, or C3. In addition, 10% of patients have anti-CFH antibodies. While the risk of post-transplant recurrence is less than 1% in Stx-HUS patients, it is approximately 80% in CFH or IF-mutated patients, 20% in MCP-mutated patients, and 30% in patients with no mutation. Patients with anti-CFH antibodies probably also are at risk of recurrence. While MCP-mutated patients can reasonably go to transplantation, recent reports suggest that plasmatherapy started before surgery and maintained life-long may prevent recurrence in CFH-mutated patients. Four successful liver-kidney transplantation utilizing plasmatherapy in CFH-mutated children have been reported recently. In summary, the risk of post-transplant recurrence can now be approached according to genotype. Therefore, aHUS patients should undergo complement determination, screening for anti-CFH antibodies, and genotyping before transplantation. Kidney or kidney + liver transplantation with concomitant plasmatherapy need to be evaluated by prospective trials in patients with hereditary complement abnormalities.

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Renal transplantation for patients with HUS cannot be considered without careful preliminary appraisal of the risk of graft loss for recurrence. It has been demonstrated during the last decade that the risk of post-transplant recurrence was less than 1% in the post-diarrheal (D+) form of HUS, caused by infection with Stx producing *Escherichia coli* (STEC), the most frequent form in children (1). Conversely, the overall risk of recurrence was shown to be at least 50% in the aHUS (1–4). More recently, it was shown that aHUS is a disease of complement dysregulation, as approximately 60% of aHUS

patients have mutations in the genes of five proteins that regulate the complement alternative pathway and protect host cells from complement activation: CFH, MCP (or CD46), a non-circulating transmembrane protein anchored in cell membranes, IF, BF, and C3 (5, 6). In addition, approximately 10% of aHUS patients have a functional CFH deficiency due to anti-CFH antibodies (7). The complement genotype now allows a more precise approach to evaluating the risk of post-transplant recurrence in aHUS patients.

## Post-transplant course in patients with D+ HUS

In a review of the literature in 2003, only one of 118 children (0.8%) with D+ HUS had unequivocal post-transplant HUS recurrence (1). In a series of 62 Argentinian children with STEC-associated HUS, all treated with cyclosporine, no patient had post-transplant recurrence, and graft survival was similar to that of patients with dysplasia/uropathies (79% vs. 76% at 10 yr

Abbreviations: aHUS, atypical hemolytic uremic syndrome; BF, factor B; CFH, complement factor H; CMV, cytomegalovirus; ESRD, end-stage renal disease; FFP, fresh frozen plasma; HUS, hemolytic uremic syndrome; IF, factor I; IVIG, intravenous immunoglobulins; MCP, membrane factor protein; PCR, polymerase chain reaction; PE, plasma exchange; SCR, short consensus repeat; STEC, toxin producing *Escherichia coli*; Stx, Shiga toxin; thrombotic microangiopathy.

follow-up), and better than that in patients with other renal diseases (79% vs. 58%,  $p < 0.001$ ) (8). The absence of post-transplant recurrence in D+ HUS patients suggests that STEC have the predominant role in the induction of the disease. Genetic predisposition may exist, which could have a role in the susceptibility to STEC and severity of D+ HUS, but apparently not in the risk of post-transplant recurrence.

Nevertheless, the classification of patients as D+ HUS or aHUS, or as STEC-associated or non-STEC-associated HUS may be spurious or misleading. First, some patients become candidates for transplantation many years after the initial HUS episode and retrospective classification may be difficult. Second, STEC infection criteria (positive PCR for *Stx* genes in stools and/or circulating anti-lipopolysaccharides antibodies) are negative in approximately 15% of D+ HUS patients (9), leaving the physician with the concern that the patient might in fact have aHUS. Third, STEC-associated HUS patients may have no prodromal diarrhea. Such patients, if not tested for STEC, could erroneously be classified as aHUS. Fourth, gastroenteritis was the triggering event in up to 28% of aHUS patients, including patients with *CFH*, *IF*, or *MCP* mutations, in the recent French pediatric series (10). In addition, STEC positive diarrhea was the triggering event of HUS onset in two *MCP*-mutated children, one of whom had multivisceral involvement leading to death (10, 11). Considering these caveats, we recommend investigating the complement system before transplantation in patients with uncertain diagnosis of D+/STEC + HUS, by determining serum levels of C3, *CFH*, *IF*, and *BF*, measurement of *MCP* expression on mononuclear cells, and screening for anti-*CFH* antibodies and for mutations in *CFH*, *IF*, *MCP*, *BF* and *C3* (Specialized Laboratories indicated in 12).

#### Post-transplant HUS recurrence in patients with aHUS

Series in non-genotyped aHUS patients in the early 2000s (1–4) indicated that the risk of post-transplant recurrence was approximately 20% in pediatric-onset aHUS patients and at least 50% in adult-onset aHUS patients. Progress in the understanding of the mechanism of the disease now allows a much more accurate approach to this problem.

aHUS as a disease resulting from defective complement control

Complement is the main actor for the defense against microbes. The system is normally

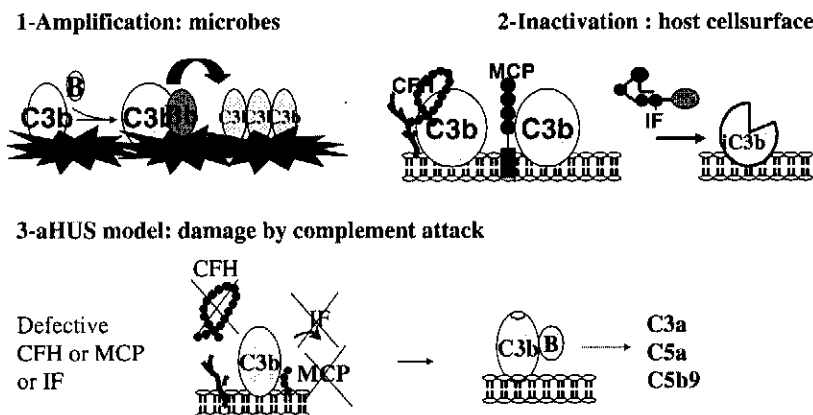
regulated so that complement activation is specifically targeted to microbe surfaces but is inhibited on intact host cells surfaces (13) (Fig. 1). The activation of complement generates the convertase C3bBb. This enzyme sets in motion an amplification reaction which converts C3 to C3b. This results in the deposition of C3b on microbe surfaces (opsonization), the formation of the terminal complex (membrane attack complex), which results in lyses of microbes. Normally, this reaction is tightly controlled at the host cell surfaces by the regulators *CFH*, *IF*, and *MCP*, which cooperates to prevent C3b deposition and activation. Mutations in these proteins induce a loss of protection of endothelial cells from complement activation (Fig. 1).

Overall post-transplant HUS recurrence in genotyped aHUS patients

In 2006–2007, the risk of HUS post-transplant recurrence was analyzed in three mostly retrospective cohorts of 280 patients with aHUS, screened for *CFH*, *IF*, or *MCP* mutations (10, 14, 15), including either only patients with pediatric onset of aHUS (10), or patients with pediatric (roughly 60%) and adult onset of aHUS (14, 15).

Approximately 50% of patients had a mutation in one of the three genes. The post-transplant course was documented in 78 patients. Post-transplant HUS recurrence was reported in 33% (10), 37% (15) and 60% (14) of grafts. Among grafts with recurrence and available data, 38% (10) to 83% (14) were lost within a period of less than one yr after recurrence.

The French pediatric series (10) illustrates the overall poor outcome of renal transplantation in aHUS children. Of the 24 renal transplants performed in 15 aHUS children, 16 (67%) failed, and 66% of patients had at least one graft failure. Of the 16 graft failures, eight (50%) were due to graft vascular thrombosis 0–45 days after surgery, and five (31%) to HUS recurrence. This high proportion of vascular thrombosis is most likely related to the thrombogenic role of complement dysregulation. Recurrence occurred in 53% (8/15) of patients, 33% (8/24) of grafts. Interestingly, five of the eight grafts (62%) with recurrence were functioning more than one yr after recurrence, while the initial HUS had induced ESRD with the first episode or after one relapse (Fig. 2). The authors suggested that graft factors such as *MCP* polymorphism could be an explanation for the apparently less severe course following HUS recurrence compared with the initial HUS in the native kidneys (10).



*Fig. 1.* Complement activation and control. aHUS is emerging as a paradigm of disease resulting from inefficient protection of the host endothelial cells surfaces in the setting of complement activation. (1) Activation of complement and covalent attachment of complement C3 to the microbial surfaces. C3b binds BF, inducing formation of the alternative C3 convertase (C3bBb) and amplification of the C3 cleavage. (2) Protection of self cell surfaces: Regulation of the cleavage of C3 is critical. In normal conditions the formation of C3 convertase is tightly controlled by CFH, MCP, and the serine protease IF. (3) In the case of aHUS, activation is uncontrolled and C3 convertase is formed resulting in formation of inflammatory mediators. CFH does not attach to surfaces through its heparin/anionic-binding sites and thus BF binds C3b. Degradation of C3b to iC3b is defective in the absence of IF and its cofactors (CFH and MCP).

#### Post-transplant HUS recurrence in patients with *CFH* mutation

Twenty to 30% of aHUS patients have *CFH* mutation, mostly heterozygous, some compound heterozygous, or homozygous. Approximately 100 different mutations have been reported (5, 6). Age at onset varies from the neonatal period to late adulthood; 70% of patients, whatever their age at onset, reach ESRD within less than one yr, either with the first episode or after one relapse (10, 15). C3 levels are decreased, although generally mildly, in most pediatric patients (10), while they were normal in 48% of patients in the Italian series including children and adults (15). Circulating CFH levels are normal in 50% (10) to 80% (15) of patients. Mutations are distributed over the entire gene, but predominate in the C terminal SCR 20 of CFH, inducing impaired CFH binding to cell surfaces. Many patients with heterozygous *CFH* mutations have normal CFH protein level but abnormal function. Therefore *CFH* mutation must be investigated even if CFH and C3 circulating levels are normal.

The post-transplant course is usually unfavorable (Table 1). In a review by Bresin et al. (14), 74% (14/19) of *CFH* mutated patients had HUS recurrence, from two days to 22 months after transplantation; 93% (13/14) of recurrences induced graft loss, most often (85.7% of recurrences) within the year after recurrence. Only one of 15 grafts with recurrence was functioning at six yr of follow-up (14). In a review of the literature, Kavanagh and Goodship also reported that 29 of 37 patients (78%) lost their

graft from recurrence (16). Among patients in the Italian Registry, five of six (83%) lost their graft from recurrence within the first post-transplant year (15). In the French pediatric cohort (10), six grafts were performed in five children, and recurrence occurred in four out of six (66%), either as full-blown HUS three days and one-month after transplantation (two patients), or as histological TMA lesions on graft biopsy performed because of increasing serum creatinine level one yr and 12 yr after transplantation (two patients). Early graft loss was due to HUS recurrence ( $n = 1$ ) or vascular thrombosis ( $n = 3$ ). Another graft with recurrence was lost 3.6 yr after transplantation, while two grafts were functioning 18 months after recurrence and 12 yr after transplantation, respectively. Therefore, outcome after recurrence in children (10) appeared less disastrous than in other series (14, 15) (Table 1).

A new type of *CFH* mutation, *CFH* hybrid gene due to gene conversion between *CFH* and *CFH* related (*CFHR1*) gene, which induces C terminal SCR 20 dysfunction, was described in 2006 (17, 18). The eight patients of one family, including three children, all died or developed ESRD, and the three of them who were transplanted lost their graft from recurrence within a year post-transplant (18). Nevertheless, of two other patients with de novo gene conversion and ESRD from HUS at the age of 11 months, one was transplanted and was free of recurrence one yr post-transplant (17).

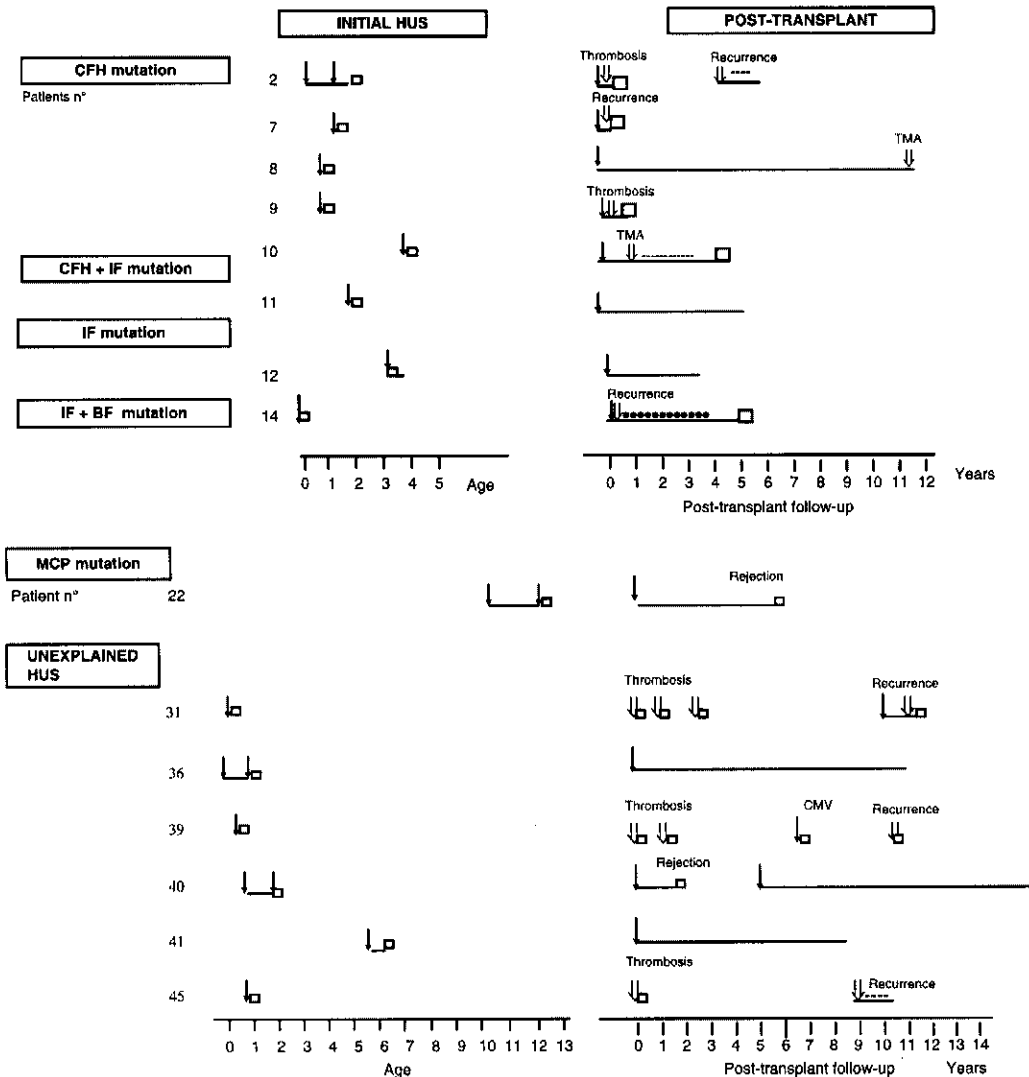


Fig. 2. Comparison of initial HUS and post-transplant course in 15 children from the French Pediatric Registry (adapted from reference 10). ↓, HUS flare; □, ESRD; ↓, Adverse event; -----, plasmatherapy efficient; ●●●●, IVIG efficient. Since the publication, a *BF* mutation associated to the *IF* mutation has been demonstrated in patient 14.

Mutation	References	Number of patients transplanted	Recurrence (% of patients)	Graft failure for recurrence within year after recurrence (% of recurrences)
<i>CFH</i>	10, 12, 14, 16, 17	34	76% (26/34)	81% (21/26)
<i>MCP</i>	10, 14, 19, 21, 22	10	20% (2/10)	1/2
<i>IF</i>	10, 23–26	8	88% (7/8)	100% (7/7)
No mutation in <i>CFH</i> , <i>IF</i> , or <i>MCP</i>	10, 14	20	30% (6/20)	83% (5/6)

Table 1. Post-transplant recurrence in aHUS patients genotyped for *CFH*, *IF*, and *MCP* (retrospective studies)

Patient 14 in Ref. 10, now known to have *IF* + *BF* mutation, is not included in the *IF*-mutated group.

In summary, the overall risk of graft loss for recurrence in patients with any kind of *CFH* mutation was approximately 80% (Table 1).

According to these series (10, 14–17), pre-transplant bilateral nephrectomy, avoidance of

calcineurin inhibitors, familial or sporadic occurrence of HUS, type of donor, *CFH* or C3 plasma levels, type and position of the mutation in the *CFH* gene, did not appear to influence the risk of HUS recurrence.

Post-transplant HUS recurrence in patients with *MCP* mutation

Ten to 15% of aHUS patients have *MCP* mutation and approximately 35 different mutations have been described, mostly heterozygous, sometimes homozygous or compound heterozygous (5, 6, 10, 15, 19–22). C3 levels are most often normal (10), but may be decreased (15). A quantitative deficiency of MCP expression on circulating mononuclear cells is observed in 80% of patients. Until now, onset earlier than one yr of age has not been reported. These patients have a relapsing course, with intervals between relapses from a few months to several years. Relapses most often have a spontaneous favorable outcome, although TMA lesions may progress. Finally, 60% (10) to 86% (15) of patients have functioning kidneys 4–5 yr after onset.

As the graft has normal MCP levels, the risk of post-transplant recurrence is logically expected to be zero. Nevertheless, of 10 MCP-mutated patients transplanted (12 grafts) (10, 15, 20, 22, 23), two (heterozygous *MCP* mutation) had recurrence (22, 23) (Table 1). One of these two patients who lost the graft for recurrence a few days after transplantation probably had a mutation in another regulator of the complement alternative pathway, suggested by persistently low C3 and BF levels (22). In the other patient, endothelial microchimerism, suggested by colonization of the graft endothelia by the recipient's MCP-deficient endothelial cells, was proposed as the explanation of recurrence (23). PE and IVIG reversed two episodes of recurrence at two months and two yr after transplantation and the graft was functioning at three and a half yr of follow-up (23 and personal communication of A. Durbach).

Post-transplant HUS recurrence in patients with *IF* mutation

Between 5% (15, 21, 24, 25) and 13% (10) of aHUS patients have an *IF* mutation, and approximately 25 different mutations have been reported, all heterozygous (5, 6). Serum C3 levels are often decreased while IF levels are fluctuating, either low or normal. Age at onset may vary from the neonatal period to adult age. Whatever the age at onset, half of patients have a rapid progression to ESRD and half recover (10, 15, 25). The reported risk of recurrence after transplantation was 100% until the most recent publication: of 11 transplants in seven patients, one was lost secondary to thrombosis and 10 from recurrence which occurred between six wk and 20 months after transplantation. All grafts with recurrence were lost within the year of recurrence (24–27) (Table 1). However, one patient in the French

pediatric series (patient 12 in Ref. 10) had no recurrence during the three yr after transplantation. Therefore, the overall risk of post-transplant recurrence in patients with *IF* mutation is 88%, and the risk of graft failure within the year after recurrence is 100% (Table 1).

The association of *IF* mutations with mutations of other complement genes, *CFH* (10), *MCP* (21, 28), *BF* or *C3* (personal communication of V. Fremeaux-Bacchi) and probably others, appears possible, and probably partly explains the HUS outcome variability. The reason why one child with a combined *CFH* and *IF* mutation had no HUS recurrence during five yr after transplantation, although he could have been considered as being at the maximum risk for recurrence, remains inexplicable (10).

Post-transplant HUS recurrence in aHUS patients with no mutation in *CFH*, *IF*, and *MCP* genes

Some patients have a *CFH*-mutated like course, with ESRD soon after onset, while others have a *MCP*-mutated like relapsing course with preserved renal function (10, 15). Overall, the risk of post-transplant recurrence in this group is 30%, with an 83% graft loss within the year after recurrence (10, 15) (Table 1).

Heterozygous mutations in *BF* have been identified in 2007, in six patients of two families, including three children (29). These mutations induce a gain of function of BF, with an increased production and decreased decay of the alternative pathway convertase (C3bBb), which induces a permanent activation of the complement alternative pathway and very low serum C3 levels. All patients progressed to ESRD. One patient was transplanted and lost the graft with recurrence (29). One *IF*-mutated patient (patient 14 in Ref. 10) with a permanent activation of the complement alternative pathway was subsequently demonstrated to have an associated *BF* mutation (personal communication of V. Fremeaux-Bacchi). This child had recurrence a few days after transplantation and lost the graft five and a half yr after recurrence.

C3 is the most recently identified susceptibility gene for aHUS. Of six patients who were transplanted (12 grafts), three had recurrence (five grafts) (personal communication of T. Goodship and V. Fremeaux-Bacchi).

Post-transplant HUS recurrence in patients with anti-*CFH* antibodies

Of three patients with anti-*CFH* antibodies who had a kidney transplant, one lost the graft with recurrence. The two others had a favorable

outcome with PEs (see below) (personal communication of A. Dragon-Durey).

### **The risk of HUS after kidney donation in living-related donors**

Because of the risk of graft loss from recurrence, living-related kidney donation has to be considered as contraindicated for patients with *CFH*, *IF*, *BF*, and *C3* mutation, highly questionable for patients with unexplained aHUS, debatable for patients with *MCP* mutation.

An additional problem has to be taken into consideration, which is the risk that the donor himself could develop HUS at sometime after kidney donation. This was reported in four donors aged 21–31 yr, who donated a kidney to one of their children or siblings and had HUS three wk to 10 months after donation (30–32). *CFH* mutation was subsequently demonstrated in one of the recipients and his donor (17, 32), while no *CFH*, *MCP*, *IF*, or *BF* mutation was found in another donor–recipient pair (personal communication of T. Goodship). The hemodynamic changes induced by unilateral nephrectomy could be the trigger for HUS in the donor with a predisposing genetic anomaly, as observed in one patient who had HUS shortly after unilateral nephrectomy secondary to a traffic accident and was discovered to have a *CFH* mutation (14).

Incomplete penetrance of the disease has been reported for all aHUS-associated mutations and approximately 50% of subjects with complement mutations are healthy, but at risk of HUS (15, 19, 20, 24, 29, 33). It is well known that many patients develop the disease during adulthood (15). One could argue that kidney donation is acceptable for the donor if he does not have the same mutation as the related recipient. Even this may not be a definitive, as the identified mutation may be only one of the risk factors shared by the recipient and donor. Some aHUS patients have a combination of *CFH*, *IF*, and *MCP* mutations (10, 28). *MCP*, *CFH*, and *CFH* linked genes polymorphisms have been shown to be risk factors for HUS, whether or not associated with identified mutations (21, 28, 29, 34–36), and these polymorphisms may be risk factors in the donor. In addition, intrafamilial genetic variability is possible, as demonstrated in two families (10 and personal communication of V. Fremeaux-Bacchi). In each family, one child with aHUS had either *CFH* or *IF* + *C3* mutation, while a sibling also with aHUS had no mutation. This shows that other unidentified genetic risk factors may be present in the patient with aHUS, and in healthy family members, who are at risk for HUS.

### **Prevention of post-transplant aHUS recurrence**

#### Bilateral nephrectomy before transplantation

An increased risk of post-transplant recurrence in patients who still had their native kidneys compared with those who have had a bilateral nephrectomy was reported in the French cohort of non-genotyped adult patients (2) but was not observed in another series (4). Among genotyped aHUS patients, although 93% (14/15) of the patients in a French pediatric group (10) had bilateral nephrectomy for hypertension before transplantation, the post-transplant recurrence rate was not lower than that reported in other genotyped cohorts (14, 15).

Therefore, it is doubtful that pretransplant bilateral nephrectomy is beneficial to prevent post-transplant recurrence.

#### Avoidance of calcineurin inhibitors

Avoidance of calcineurin inhibitors was not associated with a reduced incidence of HUS recurrence in non-genotyped cohorts (1–4) or in genotyped patients (14). HUS recurrence has been reported in aHUS patients treated by sirolimus, including one with *MCP* mutation (23).

#### Plasmatherapy

Clinicians have used plasma-based therapies for many years in aHUS patients, unknowingly treating complement abnormalities. FFP yields normal *CFH* (37), *IF* (38), *BF*, and *C3*. PE with FFP for volume substitution facilitates administering large amounts of FFP (40–100 mL/kg) without the risk of hyperproteinemia and hyperviscosity, and of volume overload and cardiac failure. In addition, PE withdraws the mutated factor(s), anti-*CFH* antibodies and potentially noxious factors such as anaphylatoxins or pro-aggregating/thrombogenic factors.

#### Plasmatherapy in patients with *CFH* mutation

Results obtained in a limited number (approximately 10) of non-transplanted *CFH* mutated-aHUS patients (39–46) have been the basis to propose plasmatherapy to prevent or rescue post-transplant HUS recurrence. Except for one patient with homozygous *CFH* mutation who remained in remission with 20 mL/kg FFP every two wk (39), most patients, whether they have homozygous (40–43), compound heterozygous (44), or heterozygous (45) *CFH* mutations, require large amounts of FFP (from 40–45 mL/kg weekly to 30 mL/kg twice weekly) to remain

in remission, which induces hyperprotidemia (> 90 g/L) and hyperviscosity. To prevent this, similar volumes of FFP are preferentially utilized with PE, either weekly, or every 4–5 wk with weekly FFP infusions in between (45). One report by Davin et al. (46) suggests that PE therapy has benefits over FFP infusions alone: two twin sisters with *CFH* heterozygous S1191L mutation in SCR 20 were successfully treated by PE with FFP, 40 mL/kg daily for 10 days, at their first episode of HUS. One twin subsequently received FFP infusions only during relapses of thrombocytopenia (17 infusions of 10 mL/kg over four months), but progressed to ESRD after four months. The other twin was maintained on PE, 40 mL/kg every two wk, intensified to daily PE for seven days during two relapses. After 44 months, her serum creatinine was 58 µmol/L.

The benefit of intensive plasmatherapy started before renal transplantation was demonstrated by the post-transplant course of the first twin (47). PE with FFP (40 mL/kg) was performed immediately before transplant surgery, and maintained afterwards, 40 mL/kg daily during seven days, then every two wk during 10 months. HUS recurrence triggered by CMV disease at 10 months was treated by intensified PE (daily during one month, then every two wk). A second recurrence at 12 months, during activation of CMV PCR, was again treated by daily PE during 10 days, then every other day during three wk. At five yr follow-up, the serum creatinine level was 122 µmol/L with weekly PE (personal communication of J. C. Davin).

Therefore, if renal transplantation is scheduled in *CFH*-mutated patients, it should be associated with administration of large amounts of FFP brought by PE started before surgery, maintained life-long, and intensified during any infectious episode (at least, intensify biological controls during infections), as this appears to prevent recurrence and graft loss.

An easier option in the future will be a human plasma-derived *CFH* concentrate, presently being developed by the Laboratoire Français du Fractionnement et des Biotechnologies, which received the European Orphan Drug designation in January 2007.

### Plasmatherapy in patients with *IF*, *MCP*, or *BF* mutation

The results of plasmatherapy in *IF*-mutated patients are scarcely documented. PE facilitated remission or improvement of HUS in three of four patients. Nevertheless, all patients progressed to ESRD within a few weeks or months

(25, 26). Three patients with post-transplant recurrence lost their grafts despite PE, but none of them was treated prophylactically (25, 26).

As MCP is not a circulating protein, no benefit is expected from plasma infusions in MCP mutated patients. Nevertheless, the patient with post-transplant recurrence attributed to endothelial microchimerism had a favorable outcome after PE (23).

The effect of plasmatherapy is not documented in *BF*-mutated patients. One may expect that very large amounts of FFP and very frequent PE will be necessary to overcome the overproduction and resistance to decay of the alternative pathway C3 convertase C3bBb.

### Plasmatherapy in patients with anti-*CFH* antibodies

PE is the optimal treatment for patients with anti-*CFH* antibodies, associated with steroids and immunosuppressive treatment (azathioprine, cyclophosphamide, mycophenolate mofetil, or rituximab) to prevent the redevelopment of antibodies after PE cessation (7, 48). We know of one patient who had a high titer of anti-*CFH* antibodies at the time of transplantation but had a successful outcome with PE (personal communication of M. A. Dragon-Durey). In a recently published observation, one child, who received pretransplant PE and rituximab to maintain anti-*CFH* antibodies at a low titer, had an uneventful post-transplant course, with excellent graft function at two yr post-transplant (49). Therefore, absence or a low level of anti-*CFH* antibodies should be obtained before transplantation with PE combined with steroids and immunosuppression, to allow low-risk transplantation.

### IVIg

Factual treatment with IVIG has occasionally been reported as efficient in aHUS patients, for instance in one child with non-genotyped aHUS (50), or to treat post-transplant recurrence in a child with *IF* and *BF* mutation (1 g/kg every three wk during five and a half yr [patient 14 in Ref. 10, in whom a *BF* mutation was subsequently demonstrated (unpublished)], or in a patient with *MCP* mutation (23). Neutralization of C5a complement activation product may be one of mechanism of action of IVIG (51).

### Liver transplantation

As *CFH* is synthesized in the liver, liver transplantation was logically proposed for patients with severe forms of aHUS and *CFH* mutation.

The first reports in three children in 2002–2005 were disappointing. The first child received a combined kidney–liver transplant at age two yr. The liver transplant failed and liver retransplantation was performed at day 26, which facilitated HUS remission, but the child had neurologic sequelae (52). The second patient, a two and a half-yr old child, had preserved renal function but severe recurrent hemolytic/thrombocytopenic episodes, and received an auxiliary liver transplant from his father. This facilitated remission of HUS, but the child died 10 months after transplantation from repeated infections, lymphoproliferative disease, and some signs of HUS recurrence during infections (53). The third patient, a two-yr old child, received a combined kidney + liver transplant, but died from primary non-function of the liver graft, with severe thrombotic and ischemic lesions suspected to be linked with the thrombogenic role of CFH dysregulation (54). At that time, it became clear that patients undergoing liver transplantation, which is known to induce complement activation, had to be treated with plasmatherapy to correct the primary complement dysregulation.

The first successful combined liver–kidney transplantation was reported by Saland et al. (55) in a five-yr old child with compound heterozygous C973Y (SCR 15) + V1197A (SCR 20) mutation. Different from the three previous reports, the procedure was covered by plasmatherapy, with one PE (about 1.25 plasma volume, 50 mL/kg FFP) immediately before surgery, and 19 mL/kg FFP infused during the operation. Enoxaparin and aspirin were administered post-operatively. No HUS recurrence has occurred and both grafts had excellent function at four yr follow-up (personal communication of J. Saland). A second child with heterozygous CFH mutation has received combined liver + kidney transplants at age four yr by the same Mount Sinai Hospital Group in New York, with a similar protocol of intensive pre- and perioperative plasmatherapy and post-operative anticoagulation. This child also had excellent function of both grafts at 10 months post-transplant (personal communication of J. Saland).

Two children with CFH heterozygous R1215Q (SCR 20) mutation were treated with a similar protocol in Helsinki. One of them had factor V Leiden mutation in addition. Both received a combined liver + kidney transplant combined with extensive plasmatherapy (PE with FFP 98 mL/kg just before surgery, FFP 36 mL/kg during operation in one child. PE with FFP: 52 mL/kg 12 h before operation, 86 mL/kg just

before operation, and 70 mL/kg between liver and kidney graft in the second child). Low molecular weight heparin was started a few hours after operation in both children, as well as long-term aspirin. Both children have normal function of both grafts and no recurrence of HUS at 15 and 18 months post-transplant, respectively (56).

Note that none of the four patients needed PE in the post-operative period, although physicians were prepared to do PE if there had been any evidence of liver dysfunction or coagulopathy.

Also note that these four patients had severe HUS, with failure of conservative treatment and/or central nervous system and ophthalmologic complications.

Therefore, combined liver–kidney transplantation combined with intensive plasmatherapy started before operation now appears as a viable therapeutic option for patients with mutations of factors synthesized in the liver: CFH, IF, BF, C3 and others to be discovered, and severe HUS, when conservative treatment is ineffective or when complications, especially neurological, are a potential for significant morbidity or mortality.

#### Recommendations in 2007

In summary, recommendations in 2007 can reasonably be as follows:

- 1) All patients with aHUS, and to our opinion also patients with an uncertain diagnosis of D+ / STEC + HUS, should undergo complement factors determination (C3, CFH, IF, BF, and MCP expression), screening for anti-CFH antibodies, and genotyping for CFH, CFH-linked genes, IF, MCP, BF, and C3 before transplantation (12).

- 2) Living-related donors should not be recommended for aHUS patients whatever their genetic background. Nevertheless, when live related transplantation is the only possible option and the donor wishes to proceed his project, complete genotyping of the related donor should be performed. If the donor has neither the mutation of his related recipient nor any other mutation, he must be informed that there remains a possibility that he might have some unknown risk factor of developing HUS after kidney donation.

- 3) Patients with MCP mutation can reasonably undergo transplantation. For the other patients, the decision of kidney transplantation is difficult.

- 4) For patients with CFH mutation, three options are presently available (Fig. 3):

## Transplantation in hemolytic uremic syndrome

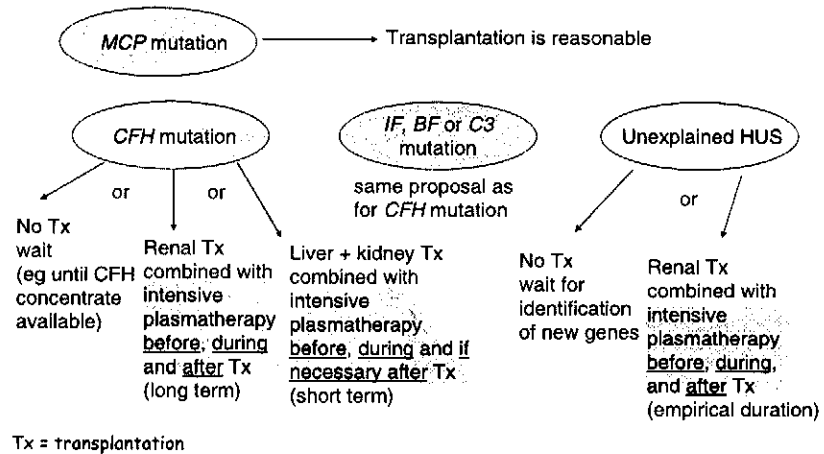


Fig. 3. Renal transplantation in aHUS patients: therapeutic options in 2007.

- First option: no transplantation, wait, for instance until CFH concentrate is available.
- Second option: kidney transplantation combined with intensive plasmatherapy: one PE with at least 40–60 mL/kg FFP just before operation, infusion of 10–20 mL/kg FFP during operation, post-operative PE, 40–60 mL/kg FFP, daily during at least one wk, progressively tapered to 1/wk, maintained life-long, and reintensified to daily PE during infectious episodes. One has to keep in mind that the efficacy of such a protocol has only been demonstrated in one child with heterozygous *CFH* mutation (47). Larger amounts of FFP and more frequent PE may be necessary in some patients, while others may require only long term FFP infusions.
- Third option: combined liver + kidney transplantation combined with intensive plasmatherapy started before operation, continued during operation and if necessary in the immediate post-operative period, associated with post-operative anticoagulation.
- The decision to consider liver transplantation in *CFH* mutated patients with preserved renal function is difficult. *CFH* concentrate infusions or antiC5 monoclonal antibodies (eculizumab) would be a less aggressive approach.

5) For patients with *IF*, *BF*, or *C3* mutation, therapeutic options similar to those for *CFH* mutated patients (except for *CFH* concentrate) have to be evaluated.

6) For patients with no identified mutation, there are two options: either wait until new genes are identified, or proceed with renal transplantation combined with intensive plasmatherapy (Fig. 3).

### Conclusion

Genotyping is of great help for proceeding with renal transplantation in aHUS patients. Renal transplantation can be performed in patients with isolated *MCP* mutations, while it is questionable for the other patients. Pre-, peri-, and post-operative plasmatherapy appears to be efficient in preventing post-transplant recurrence in *CFH*-mutated patients.

Recent success with liver + kidney transplantation combined with pre- and perioperative plasmatherapy in four patients with *CFH* mutation opens this option for patients with mutations in complement proteins synthesized by the liver. Hopefully, progress in the treatment of aHUS will prevent ESRD and reduce the number of patients who become candidates for transplantation.

### Glossary

A gene mutation is a permanent change in the DNA sequence. Gene conversion is a non-reciprocal transfer of genetic information. Hybrid gene *CFH/CFHL1* encodes a protein with the SCR 1-18 of *CFH* and the last two SCR of *CFHL1*.

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