View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Edinburgh Research Explorer

THE UNIVERSITY of EDINBURGH

Edinburgh Research Explorer

# Clinical practice guidelines for the management of atypical haemolytic uraemic syndrome in the United Kingdom

**Citation for published version:**
Taylor, CM, Machin, S, Wigmore, SJ, Goodship, THJ & working party from the Renal Association, the British Committee for Standards in Haematology and the British Transplantation Society 2010, 'Clinical practice guidelines for the management of atypical haemolytic uraemic syndrome in the United Kingdom' British journal of haematology, vol 148, no. 1, pp. 37-47., 10.1111/j.1365-2141.2009.07916.x

**Digital Object Identifier (DOI):**
10.1111/j.1365-2141.2009.07916.x

**Link:**
Link to publication record in Edinburgh Research Explorer

**Document Version:**
Preprint (usually an early version)

**Published In:**
British journal of haematology

**General rights**
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

**Take down policy**
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

OPEN ACCESS

Download date: 20. Feb. 2015

**Clinical Practice Guidelines for the management of Atypical Haemolytic Uraemic Syndrome in the United Kingdom**

C Mark Taylor, Sam Machin, Steven Wigmore, Tim H J Goodship on behalf of a working party from the Renal Association, the British Committee for Standards in Haematology and the British Transplantation Society

Corresponding Author: T H J Goodship
Institute of Human Genetics
Newcastle University
Central Parkway
Newcastle upon Tyne
NE1 3BZ

t.h.j.goodship@ncl.ac.uk

## 1. Aim, scope and audience of the guidelines

These guidelines make recommendations for the investigation and management of patients within the United Kingdom who present with features compatible with a diagnosis of atypical haemolytic uraemic syndrome (aHUS). The guidelines are intended for use by healthcare professionals involved in the management of such patients. The recommendations in the guidelines are in keeping with other guidelines recently published by the European Paediatric Study Group for HUS (Ariceta, *et al* 2008) and the Consensus Study Group for liver-kidney transplantation in aHUS (Saland, *et al* 2008). The GRADE system (http://www.gradeworkinggroup.org) has been used to classify the strength of recommendations (strong or weak) and the quality of evidence (high, moderate, low and very low) (Guyatt, *et al* 2008).

## 2. Classification, nomenclature and background

Haemolytic Uraemic Syndrome (HUS) is a disease characterised by the triad of acute renal failure, thrombocytopenia and microangiopathic haemolytic anaemia (Coombs' test negative). The demonstration of a role for enterohaemorrhagic *Escherichia coli* and verotoxin (shiga-like toxin) in D+HUS (Karmali, *et al* 1983) confirmed the differentiation between diarrhoea-associated and non-diarrhoeal forms (D-) forms (Barratt, *et al* 1987). The D- form subsequently also became known as atypical HUS (aHUS). In a small number of patients the disease was also shown to be familial (Kaplan, *et al* 1975) and/or recurrent (Kaplan 1977).

Our increased understanding of the molecular mechanisms responsible for both HUS and thrombotic thrombocytopenic purpura (TTP) has lead to the need to re-examine the classification of these diseases. A recent publication from the European Paediatric Research Group for HUS has suggested that a new classification based on aetiology be adopted (Besbas, *et al* 2006). Table 1 shows this new classification, those diseases

which might currently be considered under the heading of aHUS are highlighted. The term "aHUS" will be used throughout the rest of these guidelines and the forms which comprise this group are the focus of this document.

**2.1 Infection induced HUS**

Infection-induced HUS includes shiga and shiga-like (toxin)-producing bacteria (classically associated with D+ HUS) and *Streptococcus Pneumoniae*. Typically D+HUS is triggered by enterohaemorrhagic *Escherichia coli*, manifests as an acute self-limiting disease and >90% of childhood cases recover independent renal function spontaneously. Invasive *Streptococcus pneumoniae* produce neuraminidase that cleaves sialic acid residues from various glycoproteins, and on red cells surfaces exposes the Thomsen-Freidenreich antigen (T-antigen). Desialation is thought to predispose to a thrombotic microangiopathy (Klein, *et al* 1977).

**2.2 Disorders of complement regulation**

Disorders of complement regulation includes mutations and copy number variation in the genes encoding factor H (*CFH*), membrane cofactor protein (*MCP*), factor I (*CFI*), factor B (*CFB*), C3 (*C3*) and factor H related proteins 1-5 (*CFHR1-5*).

In ~30% of patients mutations will be found in the gene encoding the soluble complement regulator factor H (Saunders, *et al* 2006).

Autoantibodies against factor H have been reported in ~10% of aHUS patients (Dragon-Durey, *et al* 2005, Jozsi, *et al* 2007) and are associated with deficiency of factor H related protein 1(Jozsi, *et al* 2007) .

In ~10% of patients mutations are found in the widely expressed transmembrane regulator membrane cofactor protein (Richards, *et al* 2007).

In another 10% mutations are found in the serine protease factor I which is responsible for cleaving complement convertases with the aid of cofactors such as

factor H and MCP. Mutations in factor I that affect both secretion and function have been reported (Caprioli, *et al* 2006, Fremeaux-Bacchi, *et al* 2004, Kavanagh, *et al* 2005).

Activating mutations in complement factor B and C3 have also recently been reported in a small number of patients (Fremeaux-Bacchi, *et al* 2008, Goicoechea de Jorge, *et al* 2007). A hybrid complement gene which encodes a protein identical to a mutant form of factor H has also been described (Venables, *et al* 2006). The penetrance of mutations in these genes is ~50% and it has been shown that a variety of factors including copy number of other complement genes (Zipfel, *et al* 2007) and susceptibility haplotypes in factor H and MCP (Caprioli, *et al* 2003, Esparza-Gordillo, *et al* 2005) are responsible. It has also been shown that mutations in more than one complement gene can influence the predisposition to developing HUS (Esparza-Gordillo, *et al* 2006).

**2.3 ADAMTS13 Deficiency**

On rare occasions, patients presenting with the clinical phenotype of aHUS have a profound deficiency of ADAMTS13, either due to a congenital ADAMTS13 defect (eg. in young children) or acquired antibody to ADAMTS1. These patients are better described as having TTP and should be managed according to the "Guidelines on the Diagnosis and Management of the Thrombotic Microangiopathic Haemolytic Anaemias" (Allford, *et al* 2003)

**2.4 Disorders of defective cobalamine metabolism**

Disorders of intracellular cobalamine metabolism, usually Cobalamin C disease, predispose to a thrombotic microangiopathy (cblC) (Geraghty, *et al* 1992). This is characterised by methylmalonic aciduria and homocystinuria,. HUS with cblC has also been associated with a factor H mutation (Guigonis, *et al* 2005)

**2.5 Quinine induced thrombotic microangiopathy**

Quinine induced thrombotic microangiopathy is associated with antibodies against glycoproteins on platelets.

**2.6. Other diseases associated with a thrombotic microangiopathy.**

Diseases associated with a thrombotic microangiopathy where the aetiology is not so well defined includes HIV, malignancy, drugs (including cytotoxics, calcineurin inhibitors, oral contraceptives and antiplatelet agents) (Dlott, *et al* 2004), pregnancy, SLE and antiphospholipid antibody syndrome.

**3. Demography of aHUS.**

aHUS is a rare disease (incidence ~ 2 per million population per year) that affects all ages but primarily children and young adults. The incidence of aHUS in children is approximately 5 percent that of typical HUS. Updated national data on paediatric HUS established for some European countries (France, Germany, Austria and Italy) enable an estimated prevalence of aHUS of 7 per million children in the whole of the European Community.

**4. Investigations in patients presenting with aHUS**

**4.1 Measurement and interpretation of serum complement components.**

Blood should be taken for the measurement of C3, C4, factor H and factor I concentration, and for antibodies against complement components (see 4.4) before either plasma exchange or plasma infusions are commenced. C4 levels are in most cases normal. C3 levels can be either normal or low in patients with a mutation in *CFH*, *CFI* and *MCP*. The percentage of patients who have a normal C3 level in the presence of such a change ranges from 50% for *CFH* to 30% for *CFI* and 70% for *MCP* (Caprioli, *et al* 2006, Kavanagh, *et al* 2006). ~10% of patients who do not have

an identified mutation or autoantibodies to factor H will have a low C3 level suggesting that other as of yet unidentified complement components are involved.

Factor H and factor I concentrations will be normal in those patients with a mutation that does not affect secretion of the mutant protein. This includes both missense mutations and deletions (Saunders, *et al* 2007, Saunders, *et al* 2006). Factor H and factor I concentrations are lower in neonates (de Paula, *et al* 2003) and this has to be taken into account when interpreting results in this age group. There is also a large variation in factor H concentrations in normal adults and use of "normal ranges" to interpret individual results must be cautious. The majority of factor H mutations that result in impaired secretion are heterozygous and one would therefore expect that in such individuals the factor H concentration would be ~50% of normal. However, because the population normal range is so large a 50% concentration for an individual may still lie within the population normal range. Of the factor H mutations listed on the FH-HUS mutation database (www.fh-hus.org) 37% are associated with a normal factor H level and 22% a low level. For the factor I mutations listed on the same database 42% are associated with a low factor I level and 25% a normal level. As for factor H the majority of those mutations in factor I that result in impaired secretion are heterozygous.

***Recommendation.*** *In all patients presenting with clinical features compatible with a diagnosis of aHUS serum levels of C3, C4, factor H and factor I should be measured as the results guide prognosis and transplantation options.* (strong, moderate)

**4.2 FACS analysis.**

Because MCP is a transmembrane protein, FACS analysis provides a means to quickly screen for mutations that result in decreased expression. A wide range of antibodies are commercially available. Expression of MCP on peripheral blood

mononuclear cells (PBMCs) is stable at room temperature for up to 4 days. The time from venesection to analysis should be kept within this time. ~70% of *MCP* mutations are associated with decreased expression by FACS analysis. The majority of these are heterozygous but there are a few rare compound heterozygous and homozygous individuals.

***Recommendation.*** *In all patients presenting with clinical features compatible with a diagnosis of aHUS expression of MCP on PBMCs should be assessed using FACS analysis in an appropriately accredited laboratory as the results guide prognosis and late transplantation options*. (strong, moderate)

**4.3 Genetic analysis**

As mentioned previously normal levels or expression of factor H, factor I and MCP do not rule out the possibility of a normally expressed but functionally impaired mutant. Full genetic analysis is therefore recommended for all patients as the results guide prognosis and transplantation options.

In patients with low serum levels or expression of factor H, factor I and MCP in association with nonsense mutations, frameshift mutations, splice site mutations or missense mutations affecting critical residues (such as the cysteine residues in factor H responsible for forming disulphide bonds) it is probable that the abnormalities predispose to the disease. The interpretation is more difficult when the change is novel, has not been examined functionally and occurs in a region where there is no known function.

Mutations have now been described in five complement genes and it is likely that further genes will be identified. At present the turn around time for screening the genes for factor H, factor I and MCP alone is several months. For clinicians to be able to make timely decisions particularly about treatment, particularly transplantation, this

needs to be improved. New sequencing platforms are being introduced which will substantially reduce this turn around time. The Northern Molecular Genetics Service in Newcastle upon Tyne is the approved UK Genetics Testing Network laboratory for HUS (www.ukgtn.org). This laboratory has recently installed a Roche FLX System Genome Sequencer which allows ultra-rapid high-throughput sequencing. With a single read accuracy greater than 99.5% 100 million base pairs can generated in an 8 hour run. Platforms such as this will undoubtedly be the method of choice in the future for mutation screening in HUS patients.

***Recommendations.*** *Mutation screening of the genes encoding factor H, MCP, factor I, factor B and C3 should be undertaken in all patients with aHUS. This includes all historic cases who are being considered for transplantation. This should be undertaken in appropriately accredited molecular diagnostic laboratories and include appropriate techniques to detect copy number variation and hybrid genes. If mutations are found in other genes in the research setting then these should be incorporated into the molecular diagnostic portfolio*. (strong, moderate)

**4.4 Autoantibody screening**

Between 6-10% of aHUS patients have antibodies which bind to the C terminal region of factor H (Dragon-Durey, *et al* 2005, Jozsi, *et al* 2007). These can be detected with an ELISA using human factor H-coated plates to capture anti-CFH antibodies.

***Recommendations.*** *Autoantibodies against factor H should be sought in all patients with end aHUS. This should be undertaken in an appropriately accredited laboratory.* (strong, moderate)

**4.5 Rare causes of aHUS.** As mentioned previously a thrombotic microangiopathy can also be seen in association with malignancy, drugs, HIV infection, pregnancy, SLE, antiphospholipid antibody syndrome and Cobalamin C disease.

***Recommendations.*** *The possibility of these rarer forms of aHUS should be considered at presentation and appropriate investigations undertaken.* (strong, moderate)

**4.6 Measurement and interpretation of ADAMTS13 Activity**

A clinical diagnosis of aHUS is made when the predominant presenting feature is acute renal failure whereas a diagnosis of TTP is made when the predominant presenting feature is neurological. However, renal involvement is common in TTP, with proteinuria and microscopic haematuria being the most constant findings (Kennedy*, et al* 1980, Ruggenenti and Remuzzi 1990). Renal function is depressed in 40% to 80% of patients, although severe acute renal failure (which is the hallmark of aHUS) is rare (Ruggenenti and Remuzzi 1990). For this reason TTP has been closely intertwined with HUS, as evidenced by frequent use of the hybrid term HUS/TTP in the literature (Galbusera*, et al* 1999, Remuzzi*, et al* 2002a). Measurement of ADAMTS 13 activity can help to differentiate between these two clinical diagnoses. In TTP two primary mechanisms for deficiency of the ADAMTS13 activity have been identified, namely a constitutive deficiency and the presence of a circulating acquired inhibitory antibody (Furlan*, et al* 1998, Tsai and Lian 1998). In aHUS low levels of protease activity can be seen in the absence of either an inherited deficiency or anti-ADAMTS13 antibodies but both these two abnormalities can also present with a clinical phenotype of aHUS (Remuzzi*, et al* 2002a, Veyradier*, et al* 2001). It is therefore important that specific assays are undertaken to detect these two abnormalities in patients with a clinical diagnosis of aHUS in whom the presence of low levels of ADAMTS13 protease activity is detected.

***Recommendations.*** *Measurement of ADAMTS13 activity should be undertaken in patients with a clinical diagnosis of aHUS in whom other investigations do not show*

*an underlying abnormality. If ADAMTS13 activity is found to be low specific assays to detect inherited deficiency or anti-ADAMTS13 antibodies should be undertaken.* (weak, low)

**5. Management of aHUS**

**5.1 Plasma exchange/plasma infusion**.

Plasma exchange and/or plasma infusions have become empirical, first line management of a patient presenting with the features of aHUS (reviewed in (Barz*, et al* 2002, Kwon*, et al* 2008, von Baeyer 2002)). However, the information for which therapeutic strategy is most appropriate is limited. Plasma exchange is commonly undertaken daily using 1-2 plasma volumes per session in adults and 50-100 ml/kg in children. Typically plasma exchange is undertaken daily initially, the duration and frequency of treatment is then determined by the clinical response. The theoretic advantage of plasma exchange over infusion has been emphasised in childhood cases in whom the risk of rapid progression to end-stage renal failure is high (Ariceta*, et al* 2008). Plasma infusion is undertaken daily initially, but may be dose limited because of impaired renal function and hypertension. If it appears successful with reversal of the microangiopathic anaemia the dose and frequency may be reduced later to weekly or biweekly intervals. However, individual patients respond differently and some require daily plasma infusions for long periods. Apart from this and general supportive measures there is currently no specific therapy for aHUS. Genotype-phenotype correlations have helped to explain some of the variation seen in patient response. For instance patients with abnormalities in soluble regulators such as factor H respond better to plasma exchange than patients with abnormalities in the transmembrane regulator MCP (Caprioli*, et al* 2006) .

The clinical outcome for patients who present with aHUS is poor. There is an initial 25% mortality and 50% of survivors do not recover renal function (Caprioli, *et al* 2006).

***Recommendations.*** *All patients presenting with aHUS should be offered a trial of plasma exchange and/or plasma infusions.* (weak, low)

**5.2 Kidney transplantation**.

Renal transplantation is associated with an overall ~50% rate of HUS recurrence in the allograft. The outcome varies according to the underlying genetic abnormality. Renal transplantation in patients known to have either a *CFH* or *CFI* mutation has a very poor outcome with 80% of patients losing their graft to recurrent disease within two years (Bresin, *et al* 2006). In contrast transplantation in patients with defects in only the transmembrane regulator MCP have a good outcome. In this case the defective protein is replaced with the transplant and normal situation is restored. Information about the outcome of transplantation in patients with *C3* and *CFB* mutations is becoming available and suggests that the outcome of renal transplantation is also compromised by a significant risk of recurrence. There is, however, insufficient data at present to make specific recommendations for this group of patients. Likewise information about the outcome of renal transplantation alone in those patients known to have anti factor H autoantibodes is limited but does suggest again that there is a significant risk of recurrence. However, there is evidence that use of plasma exchange in combination with Rituximab can enable a satisfactory outcome (Kwon, *et al* 2008). Thus, there is a need to define the primary genetic defect in patients with aHUS, as this information is informative for therapy and disease progression. Live related renal transplantation alone is associated with the same risk of recurrence in the aforementioned groups and also a risk of the donor developing

the disease if they should carry the same mutation as the recipient (Donne, *et al* 2002).

***Recommendations***. *Renal transplantation alone is not recommended in patients known to have a factor H or factor I mutation. Patients carrying an MCP mutation, but no additional mutation in factor H, factor I, factor B and C3 or an anti-factor H autoantibody can be informed that the risk of recurrence post transplantation is low. Patients known to have a C3 or CFB mutation should be informed that current evidence suggests that there is a significant risk of disease recurrence post transplantation. Patients known to have an anti-factor H autoantibody should be treated to minimise the antibody titre before proceeding to renal transplantation. Living related renal transplantation alone should be avoided in aHUS.* (strong, moderate)

**5.3 Liver and combined liver/kidney transplantation**.

Factor H is primarily produced by the liver and liver or combined liver/kidney transplantation is therefore a logical treatment for patients known to have a factor H mutation. Combined liver/kidney transplantation has been undertaken for those patients with a factor H mutation, and one child with moderate renal damage has been treated with an isolated liver graft. This appears logical given that both factor H and factor I are produced predominantly by the liver. Whilst the initial experience with this was not favourable (Cheong, *et al* 2004, Remuzzi, *et al* 2002b, Remuzzi, *et al* 2005) more recent studies have documented a good outcome (Jalanko, *et al* 2008, Saland, *et al* 2006). This may be influenced by the use of prophylactic plasma exchange immediately prior to surgery and plasma infusion intraoperatively.

In patients who have stable renal function but disease relapses in spite of plasma therapy an isolated orthotopic liver transplant may be considered.

***Recommendations.*** *In aHUS patients with a known mutation in either factor H or factor I consideration should be given either an isolated liver or a combined liver/kidney transplant as part of an internationally coordinated clinical trial. Within the UK an advisory panel should be established to consider all patients prior to listing. Within the UK liver transplantation alone or in combination with a kidney transplant should only be undertaken in a limited number of centres with appropriate expertise.* (weak, low)

## 6. Arranging investigations for aHUS patients in the UK

Details of European laboratories which are able to undertake the aforementioned investigations are provided by Ariceta et al (Ariceta*, et al* 2008). Within the UK the UK Genetics Testic Network (http://www.ukgtn.nhs.uk/gtn/Home) approved laboratory is the Northern Molecular Genetics Service in Newcastle upon Tyne. The request form for this service is in the appendix. This gives details of sample requirements and costs. vWF cleaving crotease (ADAMTS13) assay is available from the haematology department at University College, London. A copy of the request form is in the appendix. This again gives details of sample handling.

**Table 1.** Classification of thrombotic microangiopathies adapted from (Besbas*, et al* 2006) with permission. Those conditions which might be included under the heading of aHUS are highlighted.

| | |
|---|---|
| Advanced understanding of aetiology | |
| 1.i | *Infection induced* |
| | (a) Shiga and verocytotoxin (shiga-like toxin)-producing bacteria |
| | (b) *Streptococcus pneumoniae* |
| 1.ii | *Disorders of complement regulation*, |
| | (a) Genetic disorders of complement regulation |
| | (b) Acquired disorders of complement regulation, for example anti-FH antibody |
| 1.iii | *ADAMTS13 abnormalities* |
| | (a) ADAMTS13 deficiency secondary to mutations |
| | (b) Autoantibodies against ADAMTS13 |
| 1.iv | *Defective cobalamine metabolism* |
| 1.v | *Quinine induced* |
| Aetiology not fully understood | |
| 2.i | HIV |
| 2.ii | Malignancy |
| 2.iii | Drugs |
| 2.iv | Pregnancy |
| 2.v | Systemic lupus erythematosis and antiphospholipid antibody syndrome |

## References


Allford, S.L., Hunt, B.J., Rose, P. & Machin, S.J. (2003) Guidelines on the diagnosis and management of the thrombotic microangiopathic haemolytic anaemias. *British Journal of Haematology,* **120,** 556-573.

Ariceta, G., Besbas, N., Johnson, S., Karpman, D., Landau, D., Licht, C., Loirat, C., Pecoraro, C., Taylor, C.M., Van de Kar, N., Vandewalle, J. & Zimmerhackl, L.B. (2008) Guideline for the investigation and initial therapy of diarrhea-negative hemolytic uremic syndrome. *Pediatric Nephrology*.

Barratt, T.M., Dillon, M.G., Hardisty, R.M., Levin, M., Nokes, T.J.C., Stroobant, P. & Walters, M.D.S. (1987) The role of platelets and platelet-derived growth factors in the pathogenesis of haemolytic-uraemic syndrome. In: *Recent Advances in Pediatric Nephrology* (ed. by T. Murakami, K. Kitigawa & K. Yabuta), pp. 577-580. Excerpta Medica, Amsterdam.

Barz, D., Budde, U. & Hellstern, P. (2002) Therapeutic plasma exchange and plasma infusion in thrombotic microvascular syndromes. *Thrombosis Research,* **107 Suppl 1,** S23-27.

Besbas, N., Karpman, D., Landau, D., Loirat, C., Proesmans, W., Remuzzi, G., Rizzoni, G., Taylor, C.M., Van de Kar, N. & Zimmerhackl, L.B. (2006) A classification of hemolytic uremic syndrome and thrombotic thrombocytopenic purpura and related disorders. *Kidney International,* **70,** 423-431.

Bresin, E., Daina, E., Noris, M., Castelletti, F., Stefanov, R., Hill, P., Goodship, T.H.J. & Remuzzi, G. (2006) Outcome of renal transplantation in patients with non-shiga toxin-associated hemolytic uremic syndrome: Prognostic significance of genetic background. *Clinical Journal of the American Society of Nephrology,* **1,** 88-99.

Caprioli, J., Castelletti, F., Bucchioni, S., Bettinaglio, P., Bresin, E., Pianetti, G., Gamba, S., Brioschi, S., Daina, E., Remuzzi, G. & Noris, M. (2003) Complement factor H mutations and gene polymorphisms in haemolytic uraemic syndrome: the C-257T, the A2089G and the G2881T polymorphisms are strongly associated with the disease. *Human Molecular Genetics,* **12,** 3385-3395.

Caprioli, J., Noris, M., Brioschi, S., Pianetti, G., Castelletti, F., Bettinaglio, P., Mele, C., Bresin, E., Cassis, L., Gamba, S., Porrati, F., Bucchioni, S., Monteferrante, G., Fang, C.J., Liszewski, M.K., Kavanagh, D., Atkinson, J.P. & Remuzzi, G. (2006) Genetics of HUS: the impact of MCP, CFH and IF mutations on clinical presentation, response to treatment, and outcome. *Blood,* **108,** 1267-1279.

Cheong, H.I., Lee, B.S., Kang, H.G., Hahn, H., Suh, K.S., Ha, I.S. & Choi, Y. (2004) Attempted treatment of factor H deficiency by liver transplantation. *Pediatric Nephrology,* **19,** 454-458.

de Paula, P.F., Barbosa, J.E., Junior, P.R., Ferriani, V.P., Latorre, M.R., Nudelman, V. & Isaac, L. (2003) Ontogeny of complement regulatory proteins - concentrations of factor H, factor I, C4b-binding protein, properdin and vitronectin in healthy

children of different ages and in adults. *Scandinavian Journal of Immunology,* **58,** 572-577.

Dlott, J.S., Danielson, C.F., Blue-Hnidy, D.E. & McCarthy, L.J. (2004) Drug-induced thrombotic thrombocytopenic purpura/hemolytic uremic syndrome: a concise review. *Therapeutic Apheresis and Dialysis,* **8,** 102-111.

Donne, R.L., Abbs, I., Barany, P., Elinder, C.G., Little, M., Conlon, P. & Goodship, T.H.J. (2002) Recurrence of hemolytic uremic syndrome after live related renal transplantation associated with subsequent de novo disease in the donor. *American Journal of Kidney Diseases,* **40,** E22.

Dragon-Durey, M.A., Loirat, C., Cloarec, S., Macher, M.A., Blouin, J., Nivet, H., Weiss, L., Fridman, W.H. & Fremeaux-Bacchi, V. (2005) Anti-Factor H autoantibodies associated with atypical hemolytic uremic syndrome. *Journal of the American Society of Nephrology,* **16,** 555-563.

Esparza-Gordillo, J., Goicoechea de, J.E., Buil, A., Carreras, B.L., Lopez-Trascasa, M., Sanchez-Corral, P. & Rodriguez de, C.S. (2005) Predisposition to atypical hemolytic uremic syndrome involves the concurrence of different susceptibility alleles in the regulators of complement activation gene cluster in 1q32. *Human Molecular Genetics,* **14,** 703-712.

Esparza-Gordillo, J., Goicoechea de Jorge, E., Garrido, C.A., Carreras, L., Lopez-Trascasa, M., Sanchez-Corral, P. & Rodriguez de Cordoba, S. (2006) Insights into hemolytic uremic syndrome: Segregation of three independent predisposition factors in a large, multiple affected pedigree. *Molecular Immunology,* **43,** 1769-1775.

Fremeaux-Bacchi, V., Dragon-Durey, M., Blouin, J., Vigneau, C., Kuypers, D., Boudailliez, B., Loirat, C., Rondeau, E. & Fridman, W. (2004) Complement Factor I : a susceptibility gene for atypical hemolytic-uremic syndrome. *Journal of Medical Genetics,* **41,** e84.

Fremeaux-Bacchi, V., Miller, E.C., Liszewski, M.K., Strain, L., Blouin, J., Brown, A.L., Moghal, N., Kaplan, B.S., Weiss, R.A., Lhotta, K., Kapur, G., Mattoo, T., Nivet, H., Wong, W., Gie, S., Hurault de Ligny, B., Fischbach, M., Gupta, R., Hauhart, R., Meunier, V., Loirat, C., Dragon-Durey, M.A., Fridman, W.H., Janssen, B.J., Goodship, T.H. & Atkinson, J.P. (2008) Mutations in complement C3 predispose to development of atypical hemolytic uremic syndrome. *Blood.*

Furlan, M., Robles, R., Galbusera, M., Remuzzi, G., Kyrle, P.A., Brenner, B., Krause, M., Scharrer, V., Aumann, V., Mittler, U., Solenthaler, M. & Lammle, B. (1998) Von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. *New England Journal of Medicine,* **339,** 1578-1584.

Galbusera, M., Noris, M., Rossi, C., Orisio, S., Caprioli, J., Ruggeri, Z.M., Amadei, B., Ruggenenti, P., Vasile, B., Casari, G. & Remuzzi, G. (1999) Increased fragmentation of von Willebrand factor, due to abnormal cleavage of the subunit, parallels disease activity in recurrent hemolytic uremic syndrome and thrombotic

thrombocytopenic purpura and discloses predisposition in families. The Italian Registry of Familial and Recurrent HUS/TTP. *Blood,* **94,** 610-620.

Geraghty, M.T., Perlman, E.J., Martin, L.S., Hayflick, S.J., Casella, J.F., Rosenblatt, D.S. & Valle, D. (1992) Cobalamin C defect associated with hemolytic-uremic syndrome. *Journal of Pediatrics,* **120,** 934-937.

Goicoechea de Jorge, E., Harris, C.L., Esparza-Gordillo, J., Carreras, L., Arranz, E.A., Garrido, C.A., Lopez-Trascasa, M., Sanchez-Corral, P., Morgan, B.P. & Rodriguez de Cordoba, S. (2007) Gain-of-function mutations in complement factor B are associated with atypical hemolytic uremic syndrome. *Proceedings of the National Academy of Sciences of the United States of America,* **104,** 240-245.

Guigonis, V., Fremeaux-Bacchi, V., Giraudier, S., Favier, R., Borderie, D., Massy, Z., Mougenot, B., Rosenblatt, D.S. & Deschenes, G. (2005) Late-onset thrombocytic microangiopathy caused by cblC disease: association with a factor H mutation. *Am J Kidney Dis,* **45,** 588-595.

Guyatt, G.H., Oxman, A.D., Vist, G.E., Kunz, R., Falck-Ytter, Y., Alonso-Coello, P. & Schunemann, H.J. (2008) GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *British Medical Journal (Clinical Research Ed.),* **336,** 924-926.

Jalanko, H., Peltonen, S., Koskinen, A., Puntila, J., Isoniemi, H., Holmberg, C., Pinomaki, A., Armstrong, E., Koivusalo, A., Tukiainen, E., Makisalo, H., Saland, J., Remuzzi, G., de Cordoba, S., Lassila, R., Meri, S. & Jokiranta, T.S. (2008) Successful liver-kidney transplantation in two children with aHUS caused by a mutation in complement factor H. *American Journal of Transplantation,* **8,** 216-221.

Jozsi, M., Strobel, S., Dahse, H.M., Liu, W.S., Hoyer, P.F., Oppermann, M., Skerka, C. & Zipfel, P.F. (2007) Anti-factor H autoantibodies block C-terminal recognition function of factor H in hemolytic uremic syndrome. *Blood.*

Kaplan, B.S. (1977) Hemolytic uremic syndrome with recurrent episodes: an important subset. *Clinical Nephrology,* **8,** 495-498.

Kaplan, B.S., Chesney, R.W. & Drummond, K.N. (1975) Hemolytic uremic syndrome in families. *New England Journal of Medicine,* **292,** 1090-1093.

Karmali, M.A., Steele, B.T., Petric, M. & Lim, C. (1983) Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing Escherichia coli in stools. *Lancet,* **1,** 619-620.

Kavanagh, D., Goodship, T.H.J. & Richards, A. (2006) Atypical haemolytic uraemic syndrome. *British Medical Bulletin,* **77-78,** 5-22.

Kavanagh, D., Kemp, E.J., Mayland, E., Winney, R.J., Duffield, J.S., Warwick, G., Richards, A., Ward, R., Goodship, J.A. & Goodship, T.H. (2005) Mutations in complement factor I predispose to development of atypical hemolytic uremic syndrome. *Journal of the American Society of Nephrology,* **16,** 2150-2155.

Kennedy, S.S., Zacharski, L.R. & Beck, J.R. (1980) Thrombotic thrombocytopenic purpura: analysis of 48 unselected cases. *Seminars in Thrombosis and Hemostasis,* **6,** 341-349.

Klein, P.J., Bulla, M., Newman, R.A., Muller, P., Uhlenbruck, G., Schaefer, H.E., Kruger, G. & Fisher, R. (1977) Thomsen-Friedenreich antigen in haemolytic-uraemic syndrome. *Lancet,* **2,** 1024-1025.

Kwon, T., Dragon-Durey, M.A., Macher, M.A., Baudouin, V., Maisin, A., Peuchmaur, M., Fremeaux-Bacchi, V. & Loirat, C. (2008) Successful pre-transplant management of a patient with anti-factor H autoantibodies-associated haemolytic uraemic syndrome. *Nephrology Dialysis Transplantation,* **23,** 2088-2090.

Remuzzi, G., Galbusera, M., Noris, M., Canciani, M.T., Daina, E., Bresin, E., Contaretti, S., Caprioli, J., Gamba, S., Ruggenenti, P., Perico, N. & Mannucci, P.M. (2002a) von Willebrand factor cleaving protease (ADAMTS13) is deficient in recurrent and familial thrombotic thrombocytopenic purpura and hemolytic uremic syndrome. *Blood,* **100,** 778-785.

Remuzzi, G., Ruggenenti, P., Codazzi, D., Noris, M., Caprioli, J., Locatelli, G. & Gridelli, B. (2002b) Combined kidney and liver transplantation for familial haemolytic uraemic syndrome. **359,** 1671-1672.

Remuzzi, G., Ruggenenti, P., Colledan, M., Gridelli, B., Bertani, A., Bettinaglio, P., Bucchioni, S., Sonzogni, A., Bonanomi, E., Sonzogni, V., Platt, J.L., Perico, N. & Noris, M. (2005) Hemolytic uremic syndrome: a fatal outcome after kidney and liver transplantation performed to correct factor h gene mutation. *American Journal of Transplantation,* **5,** 1146-1150.

Richards, A., Kathryn Liszewski, M., Kavanagh, D., Fang, C.J., Moulton, E., Fremeaux-Bacchi, V., Remuzzi, G., Noris, M., Goodship, T.H. & Atkinson, J.P. (2007) Implications of the initial mutations in membrane cofactor protein (MCP; CD46) leading to atypical hemolytic uremic syndrome. *Molecular Immunology,* **44,** 111-122.

Ruggenenti, P. & Remuzzi, G. (1990) Thrombotic thrombocytopenic purpura and related disorders. *Hematology/Oncology Clinics of North America,* **4,** 219-241.

Saland, J.M., Emre, S.H., Shneider, B.L., Benchimol, C., Ames, S., Bromberg, J.S., Remuzzi, G., Strain, L. & Goodship, T.H.J. (2006) Favorable long-term outcome after liver-kidney transplant for recurrent hemolytic uremic syndrome associated with a factor H mutation. *American Journal of Transplantation,* **6,** 1948-1952.

Saland, J.M., Ruggenenti, P., Remuzzi, G. & (2008) Liver-kidney transplantation to cure atypical hemolytic uremic syndrome. *Journal of the American Society of Nephrology*.

Saunders, R.E., Abarrategui-Garrido, C., Fremeaux-Bacchi, V., Goicoechea de Jorge, E., Goodship, T.H.J., Trascasa, M.L., Noris, M., Castro, I.M.P., Remuzzi, G., Rodriguez De Cordoba, S., Sanchez-Corral, P., Zipfel, P.F. & Perkins, S.J. (2007)

The interactive factor H-atypical haemolytic uraemic syndrome mutation database and website: Update and integration of membrane cofactor protein and factor I mutations with structural models. *Human Mutation,* **28,** 222-234.

Saunders, R.E., Goodship, T.H.J., Zipfel, P.F. & Perkins, S.J. (2006) An interactive web database of factor H-associated hemolytic uremic syndrome mutations: insights into the structural consequences of disease-associated mutations. *Human Mutation,* **27,** 21-30.

Tsai, H.M. & Lian, E.C. (1998) Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. *New England Journal of Medicine,* **339,** 1585-1594.

Venables, J.P., Strain, L., Routledge, D., Bourn, D., Powell, H.M., Warwicker, P., Diaz-Torres, M.L., Sampson, A., Mead, P., Webb, M., Pirson, Y., Jackson, M.S., Hughes, A., Wood, K.M., Goodship, J.A. & Goodship, T.H. (2006) Atypical haemolytic uraemic syndrome associated with a hybrid complement gene. *PLoS Medicine,* **3,** e431.

Veyradier, A., Obert, B., Houllier, A., Meyer, D. & Girma, J.P. (2001) Specific von Willebrand factor-cleaving protease in thrombotic microangiopathies: a study of 111 cases. *Blood,* **98,** 1765-1772.

von Baeyer, H. (2002) Plasmapheresis in thrombotic microangiopathy-associated syndromes: review of outcome data derived from clinical trials and open studies. *Therapeutic Apheresis,* **6,** 320-328.

Zipfel, P.F., Edey, M., Heinen, S., Jozsi, M., Richter, M., Misselwitz, J., Hoppe, B., Routledge, D., Strain, L., Hughes, A.E., Goodship, J.A., Goodship, T.H.J. & Skerka, C. (2007) Deletion of complement factor H related genes CFHR1 and CFHR3 is associated with an increased risk of atypical hemolytic uremic syndrome. *PLoS Genetics,* **3,** e411. .

**Appendix**

CPA Accredited Laboratory

## *Complement genotyping request form*

**Patient information:**

Surname……

Forename……..

Sex……. Date of Birth (dd/mm/yy)……..…….

Address……..

……

……ZIP or Postcode……..

Hospital……..

Hospital number……

Other identifier (e.g. NHS number, social security number)

……..

**Referring Clinician (person to whom result will be sent):**

Name……..

Address……

……

……ZIP or Postcode……

e-mail address……

**To whom should the invoice be addressed (e.g. referring clinician, healthcare provider)?**

……...……

## Clinical Information:

(where available please include the levels of any complement components measured such as C3, C4, Factor I, Factor B, Factor H or if any genetic testing for aHUS has been carried out elsewhere. Please also include a pedigree if appropriate)

## Disease code:

Atypical haemolytic uraemic syndrome ☐
MPGN (membranoproliferative glomerulonephritis) ☐
*please state which type (I, II or III)*
D+HUS (diarrhoeal associated haemolytic uraemic syndrome) ☐
TMApt (post transplant thrombotic microangiopathy) ☐
HELLP (haemolysis, elevated liver enzymes, low platelets) ☐
Macular degeneration ☐

## Tests required:

**Please note that payment must be received prior to testing for non-NHS patients (please contact Hazel.Forrest@nuth.nhs.uk for more information on payment)**

| | Turnaround (working days) | Cost (£) (please tick) | Test required |
|---|---|---|---|
| Serum complement screen (C3, C4, factor H, factor I)* | 40 | 90 | ☐ |
| CD46 expression by FACS* | 40 | 35 | ☐ |
| *CFH* gene (exons 18-23) | 40 | 480 | ☐ |
| *CFH* gene (exons 1-17) | 40 | 320 | ☐ |
| *CFI* gene (exons 1-13) | 120 | 600 | ☐ |
| *CD46* gene (exons 1-13) | 120 | 600 | ☐ |
| Test for known mutation | 10 | 150 | ☐ |
| Factor H auto-antibodies (on a research basis only at present) | | | ☐ |

*Serum screen and FACS analysis carried out at Immunology, RVI, Newcastle upon Tyne.

## Samples required:

One 10 ml EDTA and one 5 ml clotted sample should be taken and each labelled with surname, forename and date of birth. The samples should be sent by courier (outside UK) or first class post (within UK) in a secure container (at ambient temperature) to:

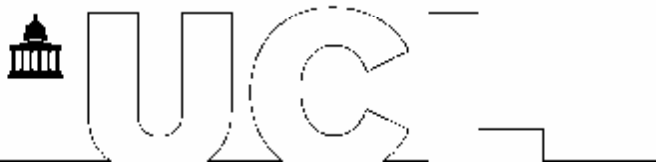
Dr Lisa Strain
Northern Molecular Genetics Service
Institute of Human Genetics
The International Centre for Life
Central Parkway
Newcastle upon Tyne
NE1 3BZ
United Kingdom

## Date of sample (dd/mm/yyyy):……..……

## Related links:

aHUS mutation database: http://www.fh-hus.org/
Genetests: http://www.genetests.org/ Lab ID 777
UK Genetic Testing Network: http://www.ukgtn.org/gtn/

**UCL HAEMATOLOGY DEPARTMENT**
HAEMOSTASIS RESEARCH UNIT



# vWF Cleaving Protease (ADAMTS13) Assay request form

Patient Name …….

Hospital Number…… Date of Sample Collection ……

Date of Birth…… Consultant ordering the assay……..

Date of last plasma infusion or exchange ……..
Assay required for Diagnosis/Clinical Management **YES / NO**
NHS Patient **OR** PRIVATE Patient **(please circle)**
This patient is registered in the Rituximab Study **YES / NO**
The patient is part of the Pneumococcal HUS Study **YES / NO**
The patient is part of the Kings Sickle Study **YES / NO**

**Full address for Report:**
……
……..
……..

**Institution's Order No.:**

**Name & Address for Billing** (if different to above):

We will assay ADAMTS13 by the collagen binding method or a peptide substrate assay, as available (and an inhibitor test if the activity is low).
If additional types of ADAMTS13 assay are required (for which additional charges will be made), please telephone and discuss.

**SAMPLE REQUIREMENTS**:

- **Collect 5ml citrated blood and ship immediately, to arrive within 4hr, OR:**
- Centrifuge at 2,000g for 15 min and (avoiding the buffy coat) pipette into 2-3x 0.5ml aliquots, freeze at –70°C and ship **on dry ice (in a suitably vented container).**

Contact the Haemostasis Research Unit to arrange delivery. The sample must arrive between 09.00-16.00 Monday-Friday (avoid public holidays!) with a copy of this form. The Department is closed outside these times.

**Delivery Address:** Haemostasis Research Unit, Haematology Dept, University College London, 1st floor, 51 Chenies Mews, London WC1E 6HX

**Please telephone Dr Ian Mackie or other HRU staff (020 7679 6416/6421/6423) before sending sample to ensure that it is processed. (E-mail: i.Mackie@ucl.ac.uk)**