A classification

of hemolytic uremic syndrome and thrombotic thrombocytopenic purpura and related disorders

N Besbas[1], D Karpman[2], D Landau[3], C Loirat[4], W Proesmans[5], G Remuzzi[6,11], G Rizzoni[7], CM Taylor[8], N Van de Kar[9] and LB Zimmerhackl[10] from the European Paediatric Research Group for HUS

[1]Hacettep University, Ankara, Turkey; [2]Lund University, Lund, Sweden; [3]Soroka Medical Centre, Beer Sheva, Israel; [4]Hôpital Robert Debré, Paris, France; [5]University Hospital, Leuven, Belgium; [6]Mario Negri Institute for Pharmacological Research, Bergamo, Italy; [7]Ospedale Bambina Gesu, Rome, Italy; [8]Birmingham Children's Hospital, Birmingham, UK; [9]UMC St Radboud, Nijmegen, The Netherlands and [10]University of Innsbruck, Innsbruck, Austria

The diagnostic terms hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) are based on historical and overlapping clinical descriptions. Advances in understanding some of the causes of the syndrome now permit many patients to be classified according to etiology. The increased precision of a diagnosis based on causation is important for considering logical approaches to treatment and prognosis. It is also essential for research. We propose a classification that accommodates both a current understanding of causation (level 1) and clinical association in cases for whom cause of disease is unclear (level 2). We tested the classification in a pediatric disease registry of HUS. The revised classification is a stimulus to comprehensive investigation of all cases of HUS and TTP and is expected to increase the proportion of cases in whom a level 1 etiological diagnosis is confirmed.

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Correspondence: *CM Taylor, Department of Nephrology, Birmingham Children's Hospital, Birmingham B4 6NH, UK. E-mail: cm.taylor@bch.nhs.uk*

[11]Consultant to the European Paediatric Research Group for HUS.

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New concepts of causation suggest an improved diagnostic classification of hemolytic uremic syndrome (HUS)[1] and thrombotic thrombocytopenic purpura (TTP),[2] two clinically defined syndromes with the shared pathology of thrombotic microangiopathy (TMA).[3,4] Broadly, subgroups of HUS/TTP can be diagnosed at two levels. The first is by etiology, the second by clinical features or associations. The proposed classification is therefore split into two sections: part 1 in which concepts of etiology are reasonably advanced, and part 2 where clinical associations are used to describe patients for whom the cause remains unclear (Table 1). The division is admittedly arbitrary, and advancing knowledge is expected to redistribute cases from part 2 to 1. Future revisions will be needed. Subgroups re-defined by etiology often have a subgroup-specific prognosis and require specific therapies.

PROPOSED CLASSIFICATION: PART 1, ETIOLOGY ADVANCED

Shiga and verocytotoxin (shiga-like toxin)-producing bacteria

The major cause of HUS in childhood is infection with verocytotoxin (shiga-like toxin)-producing bacteria, usually enterohemorrhagic *Escherichia coli* (VTEC/STEC),[5] and in some tropical regions *Shigella dysenteriae type I*.[6,7] Verocytotoxin-producing *Citrobacter freundii*, has also been reported.[8] In America and the UK most cases are associated with *E. coli* serotype O157:H7,[9–12] while other serotypes such as O26, O111, O103, and O145 are increasingly reported in Europe and elsewhere.[13–15] The epidemiology has been extensively studied. Patients are usually pre-school children.[16–18] VTEC strains produce various toxins, the major ones being verocytotoxin-1 (Stx1) and verocytotoxin-2 (Stx2). Verocytotoxin-1 differs by one amino acid from Shiga toxin produced by *Shigella dysenteriae type 1*. Verocytotoxin-2 has multiple variants that are closely related to each other, but have 55–60% homology to verocytotoxin-1. HUS is mostly caused by verocytotoxin-2-producing strains.[11,14,15]

The disease begins after an incubation of 4–7 days with abrupt onset of diarrhea, usually bloody, with abdominal

Table 1 | Classification of HUS, TTP, and related disorders

Part 1: etiology advanced	
1.i	*Infection induced*
	(a) Shiga and verocytotoxin (shiga-like toxin)-producing bacteria; enterohemorrhagic *Escherichia coli*, Shigella dysenteriaen type 1, *Citrobacter*
	(b) *Streptococcus pneumoniae,* neuraminidase, and T-antigen exposure
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	*von Willebrand proteinase, ADAMTS13 deficiency*
	(a) Genetic disorders of ADAMTS13
	(b) Acquired von Willebrand proteinase deficiency; autoimmune, drug induced
1.iv	*Defective cobalamine metabolism*
1.v	*Qinine induced*
Part 2: Clinical associations: etiology unknown	
2.i	HIV
2.ii	Malignancy, cancer chemotherapy and ionizing radiation
2.iii	Calcineurin inhibitors and transplantation
2.iv	Pregnancy, HELLP syndrome and oral contraceptive pill
2.v	Systemic lupus erythematosis and antiphospholipid antibody syndrome
2 vi	Glomerulopathy
2.vii	Familial, not included in part 1
2.viii	Unclassified

FH, factor H; HELLP, HEmolytic anemia, elevated Liver enzymes, and Low Platelets; HIV, human immunodeficiency virus; HUS, hemolytic uremic syndrome; TTP, thrombocytopenic purpura.

pain. Microangiopathic hemolytic anemia, thrombocytopenia and acute oliguric renal failure occur 2–10 days later. The diagnosis of VTEC infection is made on stool culture, identification of toxin in the stools, or by serological response to the relevant O-serotype. Verotoxin itself is implicated in the pathogenesis, both from the epidemiology and from laboratory models. A prothrombotic state evolves prior to the acute renal failure,[19] and the specific pathological finding is glomerular thrombosis.[20,21]

With supportive therapy the mortality in children is $<5\%$ (*Shigella dysenteriae type 1* excepted). Approximately 75% appear to make a full recovery when assessed up to 5 years after onset.[22] Relapse is extremely rare.

Streptococcus pneumoniae, neuraminidase, and T-antigen exposure

This is a distinctive disorder complicating pneumococcal infection, usually septicaemia, pneumonia with empyema, or meningitis with subdural collections.[23] Patients, usually <2 years old, present with marked microangiopathic hemolytic anemia. The acute mortality is about 25%.[16] HUS relapse has not been described.

Pneumococcal neuraminidase has been identified in plasma.[24] It causes de-sialation of the glycocalyx of many cells exposing the Thomsen–Friedenreich (T-) antigen that is normally covered by sialic acid.[25] T-antigen exposure on red cells is detected using the lectin *Hypogeae*. An immunoglobulin M cold antibody occurring naturally in human serum causes polyagglutination *in vitro.* Unlike other forms of HUS there is a positive Coomb's test. T-anti-T interaction on red cells, platelets, and endothelium was thought to explain the pathogenesis, but the role of the anti-T cold antibody *in vivo* is questionable.[26]

Genetic disorders of complement regulation

Mutations in the genes for complement factor H (FH), factor I (FI), and membrane co-factor protein (MCP), also known as CD46, are associated with HUS.

FH, which is abundant in plasma, binds to polyanionic sites on host cells surfaces. Here it captures C3b generated by the alternative pathway and prevents formation of the C3 convertase, C3bBb, by factor B. This prevents amplification of the complement cascade and is a major reason why host cell surfaces are normally defended from alternative pathway activity.[27] FH is known to bind to vascular endothelium, erythrocytes, and platelets.[28–30]

FH mutations have been found in a fifth of families with HUS and in about 8% in sporadic cases.[31,32] Most patients have heterozygous mutations that affect the C-terminal, reducing binding to C3b/C3d, heparin,[33] and endothelial cells.[29,34,35] Mutations leading to truncation of the FH molecule or the inability to be exported from the endoplasmic reticulum have also been described.[36] An association between three frequent single nucleotide polymorphisms of FH and susceptibility to HUS has been suggested.[37,38]

The phenotype is variable and HUS may present at any age, although homozygous or compound heterozygous FH-deficient patients are more likely to manifest HUS in early life.[39] The plasma concentration of C3 may be normal or persistently low. Striking features include severe hypertension, a high risk of relapse, and poor prognosis, 50% of cases progressing to end-stage renal failure. The risk of graft loss after transplantation approaches 80%, mostly reflecting disease recurrence.[40,41]

Mutations have been found in the genes encoding both FI[42,43] and MCP (CD46).[44,45] FI, a co-factor for FH, cleaves C3b interrupting the complement cascade before the generation of the anaphylotoxin C5a and the membrane attack complex C5b–C9 (reviewed by Zipfel *et al.*[46]). Complement C5a and the membrane attack complex play an essential pathogenetic role in certain laboratory models of HUS [47,48]

FI circulates in plasma and, using FH, MCP, or CR1 as a co-factor, cleaves C3b to iC3b, or, using C4-binding protein, MCP or CR1 as a co-factor, cleaves C4b. All HUS patients so far described except one [43] are heterozygous for stop codons that truncate protein production, and plasma concentrations of FI may be reduced. MCP, a membrane-bound regulator expressed in glomerular endothelium also acts as a co-factor for the cleavage of C3b and C4b by FI. Heterozygous and homozygous MCP mutations have been identified in more

than 15 cases. These are predicted to cause loss of C3b and C4b recognition or loss of the C-terminal of the protein. Reduced expression on nucleated cells has been shown in some patients. Compared to FH or FI mutations, those with MCP abnormalities appear less likely to have disease recurrence after transplantation, probably because the donor organ retains normal MCP expression.

The diagnosis requires genetic expertise. A low C3 cannot be relied on to indicate deficient function of a membrane-bound complement regulator, nor does a normal plasma concentration of FH or FI exclude the possibility of a functional defect. Conversely, low C3 is a positive indicator, and in families with HUS those with hypocomplementemia are at increased risk of developing the disorder.[49] The fact that three different regulator abnormalities have been found so far supports the concept that failure to regulate complement on host tissues is etiologically relevant. It raises the possibility that other regulatory defects, either individual or compound,[50] will be found.

Acquired disorders of complement regulation

Evidence of immunoglobulin G autoantibodies to complement FH has been reported in three children with relapsing HUS.[51] The plasma concentration of FH, and the FH gene were normal. FH autoantibody was identified by enzyme-linked immunosorbent assay, and shown to interfere with FH binding to the C3bBb convertase. These children also had antinuclear antibody but no other autoantibodies. Complement C3 was low in two cases as was factor B indicating alternative pathway activation. This strongly suggests that an acquired defect in complement regulation can mimic the inherited pattern outlined above.

Genetic disorders of von Willebrand factor-cleaving protease (ADAMTS13)

The finding of unusually large multimeric forms of von Willebrand factor (vWF), similar in size to those secreted by cultured endothelial cells *in vitro,* in the plasma of patients with HUS/TTP and chronic relapsing TTP was first reported by Moake *et al.*[52,53] These large multimers cause platelet agglutination at high shear stress. In 1997, Furlan *et al.*[54] identified severe deficiency ($<7\%$ of normal plasma activity) of a specific protease (vWFCP) that cleaves multimeric vWF protein in patients with chronic relapsing TTP. This was confirmed in a large group of patients with familial and non-familial TTP.[55,56] The vWFCP deficiency was shown to be either constitutional or due to autoantibodies. Lower activity occurs in liver disease, cancers, chronic inflammatory and metabolic conditions, pregnancy and in the newborn, but not the extremely low levels associated with TTP.[57,58]

vWFCP cleaves multimeric vWF released from the endothelium and platelets into monomeric subunits at position 842–843. It has been purified, the gene sequenced,[59,60] and shown to be a member of the ADAMTS family of metalloproteases and designated ADAMTS13. ADAMTS is an acronym for '*a disintegrin-like and metalloprotease with thrombospondin type I repeats*'. ADAMTS13 is encoded on chromosome 9q34 and patients with familial relapsing TTP have mutations throughout the gene.[61–64] vWFCP is produced by hepatic stellate cells[65–67] and has a plasma half-life of 2–3 days.[68] Several assays of vWFCP activity and vWFCP-inhibiting autoantibodies (see below) have been developed.[69] Although reliable and comparable, they are labor intensive and specialized.

Chronic relapsing TTP due to inherited vWFCP deficiency presents with thrombocytopenia and hemolytic anemia usually in the neonatal period.[63,70] Later, recurrent hemolysis and thrombocytopenia recur at intervals usually every 3rd–4th week. About 50% of the patients with vWFCP activity below 5% of normal have their first attack before the age of 5 years. In the others the diagnosis is made in adulthood,[71] and not all patients present with the complete diagnostic criteria of HUS/TTP. If diagnosed early, chronic relapsing TTP/HUS due to constitutional vWFCP deficiency can be reversed or prevented by infusion of fresh-frozen or virus inactivated plasma or at 2–3 weeks interval without concurrent plasmapheresis.

Acquired disorders of vWF-cleaving protease

Severely decreased vWFCP activity in patients with acute acquired, non-familial TTP is mostly due to inhibitory immunoglobulin G autoantibodies.[55,56] These are found in 48–80% of adult patients and are transient or intermittent in the majority. This form of TTP/HUS is mainly seen in adults and is usually a single acute episode, although recurrences have been reported in 11–36% of patients.

Historically, patients with untreated TTP had a mortality approaching 90%, and plasma exchange using fresh-frozen plasma reduced this to 20%.[72] Patients with vWFCP deficiency due to autoantibodies usually respond to intensive plasmapheresis but may require additional immunosuppression or even splenectomy. Rituximab, the monoclonal antibody against CD20 on B lymphocytes is also effective.[73–75]

Some cases of acquired ADAMTS13 deficiency have been linked to the platelet inhibitors ticlopidine and clopidogrel. Ticlopidine-associated TTP[76,77] occurs in one per 1600–5000 patients treated, usually within 1 month of starting. Besides the hemolytic anemia and thrombocytopenia, 75% of cases developed neurological manifestations, and 30% renal insufficiency. Plasmapheresis reduces the mortality.[77] Autoantibodies inhibiting ADAMTS13 have been detected in ticlopidine-associated TTP.[78] Clopidogrel-associated TTP usually presents within 2 weeks of starting the drug, is more prone to recurrence than with ticlopidine, and requires more plasma exchanges. The incidence of clopidogrel-associated TTP is similar to the general population (about 3.7 cases per million) making a causal relation less certain. However, antibody-induced ADAMTS13 deficiency has been reported in two cases.[79]

Defective cobalamin metabolism

This autosomal recessive form of HUS is attributed to an inborn error of cobalamin-C metabolism.[80] The biochemical characteristics of cobalamin-C deficiency are hyperhomocysteinemia and methylmalonic aciduria. High levels of homocysteine might be responsible for the vascular manifestations.

Patients with cobalamin-C deficiency usually present in the early days and months of life with failure to thrive, poor feeding, and vomiting. Rapid deterioration occurs due to metabolic acidosis, gastrointestinal bleeding, hemolytic anemia, thrombocytopenia, severe respiratory and hepatic failure, and renal insufficiency.[81,82] It is likely that some die undiagnosed. Besides the early fulminant course, a more protracted disease can manifest later in childhood and adolescence.[83–85] Renal biopsy showed a chronic TMA. Serum homocysteine can be 10 times normal values and urinary methylmalonic acid markedly increased but correct with daily hydroxycobalamin administration.[85]

Quinine

Quinine, whether as a medication or food additive, is associated with TTP/HUS.[86,87] In a series of HUS/TTP patients 11% reported taking quinine compared to 6% taking other drugs.[88] In sensitized patients the typical clinical pattern is an abrupt onset of chills, myalgia, vomiting, and oliguria immediately after quinine exposure. Anemia is often mild. Patients can be shown to have antibodies that recognize different glycoprotein epitopes on platelets, red cells, and leukocytes.[89,90] This interaction is quinine dependent, suggesting that a neoantigen is formed. In platelets, the antibody has been found to cross-react with glycoprotein IIb/IIIa and sometimes Ib/IX. The disorder is comparatively mild and remits if plasma exchange is started early enough and quinine avoided. ADAMTS13 plasma activity is typically normal.

PROPOSED CLASSIFICATION: PART 2, CLINICAL ASSOCIATIONS; ETIOLOGY UNKNOWN

Human immunodeficiency virus

Human immunodeficiency virus (HIV) positivity and acquired immune deficiency syndrome are risk factors for renal disease[91] and HIV nephropathy can include HUS. HUS appears more prevalent in the HIV population[92] and has a high mortality in advanced acquired immune deficiency syndrome. In one series it occurred in a third of HIV-infected adults and was the most frequent cause of acute or rapidly progressive renal failure.[93] HIV-associated HUS/TTP is rarer in childhood.[94] Exceptionally, HIV-positive patients develop other forms of HUS, for example one patient had complete deficiency of vWF-cleaving protease ADAMTS13 having developed an antiprotease autoantibody,[95] but in the majority alternative explanations of causation have not been sought. It is assumed that the virus directly affects the endothelium causing TMA as has been shown in a primate model.[96]

Malignancy, cancer chemotherapy, and ionizing radiation

Disseminated carcinoma, usually gastric,[97] prostatic,[98] or colon,[99] has been associated with HUS/TTP. In the registry of the Lombardi Cancer Research Center, Georgetown University, Washington DC, 85 patients met the criteria of hemolysis, thrombocytopenia, and serum creatinine above 1.6 mg/dl.[100] Eighty nine percent of them had adenocarcinoma, 26% being gastric cancer. HUS/TTP developed before cancer was diagnosed in a third of patients giving credence to the primary association. However, all except one received mitomycin at some point and it is unclear in many cases whether the association is directly with malignancy or with drugs used in its treatment. The same argument can be raised with irradiation. Infectious complications of cancer such as cytomegalovirus infection[101] and human herpes virus-6[102] may also play a role.

Mitomycin, an antimitotic used mainly in gastric and breast cancer, is associated with HUS and histological features of TMA.[103] Some cases improve with steroids and plasma exchanges.[104] Cattell[105] was able to induce TMA by injecting mitomycin into the renal artery of rats, supporting a causative role. Gemcitabine, a related anticancer drug, used for pulmonary, pancreatic, and urothelial carcinoma, is also thought to be the cause of HUS/TTP.[106,107]

Radiation nephropathy presents with hypertension proteinuria or renal impairment up to a year after exposure. Cases with superadded microangiopathic hemolytic anemia typical of HUS are rare. The renal cells most sensitive to ionizing radiation are glomerular endothelium and tubular epithelium. In radiation nephropathy, electron microscopy shows that glomerular endothelial cells are swollen, separated from the basement membrane by electron-lucent material or lost entirely. A late finding is fibrinoid necrosis and thrombosis of arteries and arterioles. This resembles TMA adding credence to the association. Modern concepts of pathogenesis have not been applied.

Calcineurin inhibitors and transplantation

HUS/TTP occurs after transplantation of liver,[108] kidney,[109] heart, kidney-pancreas,[110] and bone marrow, and with both cyclosporin-A and tacrolimus immunosuppression. In a review of 188 kidney transplants Zarifian *et al.*[111] calculated that incidence of TMA associated with cyclosporin-A was 14%, and with tacrolimus between 1 and 5%. With tacrolimus the clinical severity is very variable and drug levels do not predict development of TMA.[112] Dose reduction or changing one calcineurin antagonist for another[113] sometimes results in recovery and suggests a causative role. These drugs exert both direct and endothelin-1-mediated vasoconstriction that reduces renal plasma flow and perhaps lead to prothrombotic changes in endothelium. However, the pathophysiology is speculative. Plasma activity of ADAMTS13 has not been measured in this setting.

HUS/TTP can occur up to several months after hematopoietic stem cell (bone marrow) transplantation. In a review, Moake and Byrnes[114] point out that conditioning regimes with total body radiation and cyclophosphamide, and the use

of cyclosporin for graft-versus-host disease prophylaxis, make it impossible to determine whether any of these had a pathogenic role. George JI *et al.*[115] conclude that the association between bone marrow transplantation and HUS/TTP is rare and that all in their series had evidence of underlying sepsis. The pathogenesis has not been convincingly shown. ADAMTS13 activity has been found to be decreased in patients undergoing allogeneic bone marrow transplantation but not to the very low levels associated with TTP, and in some the activity is normal.

It is unclear whether calcineurin inhibitors should be avoided in transplanting HUS patients from diagnostic subgroups known to have a high risk of relapse. In one report cyclosporin-A and tacrolimus were not associated with HUS recurrence.[41] Sirolimus is of doubtful benefit[116] as HUS recurred in two children treated with sirolimus while avoiding calcineurin inhibitors.[117]

Pregnancy, HELLP syndrome, and oral contraceptives

TTP is more prevalent in women than in men, and occurs more often in childbearing years. An association with the use of the oral contraceptive pill remains speculative, but an association with pregnancy is clear.[118] HUS/TTP may present at any time during pregnancy but mostly in the last trimester and about the time of delivery. It is sometimes difficult to distinguish it from pre-eclampsia. Cases have been described in which TTP recurs in subsequent, although not always consecutive pregnancies. Patients with previous HUS/TTP not associated with pregnancy may or may not relapse when pregnant.

Comprehensive investigation of the cause of pregnancy-associated HUS/TTP has seldom been undertaken. A modest reduction in ADAMTS13 activity is induced by estrogens and occurs normally in the last trimester of pregnancy, but not to the very low levels observed in TTP.[119] However, a few cases of pregnancy-associated HUS/TTP have severely reduced protease activity, with or without an inhibitor and the presence of ultralarge vWF multimers in plasma.[120–123] Equally there are cases in which the protease is normal. There are no reports of complement activation in this group.

HELLP syndrome (HEmolytic anemia, elevated Liver enzymes, and Low Platelets) is also a disorder of the last trimester or parturition, and patients may have features of pre-eclampsia. The blood film typically has evidence of microangiopathic hemolytic anemia with fragmented red blood cells. Von Willebrand protease activity is reduced more than is seen in normal pregnancy, but again not to the low levels seen in TTP and without ultralarge vWF multimers in plasma.[119] Whether the moderately reduced ADAMTS13 activity plays any role in the pathogenesis is unclear. One might predict that pregnancy, and pre-eclampsia in particular, would exacerbate a prothrombotic state and add to the causation of HUS/TTP, perhaps providing a 'second hit.'

Systemic lupus erythematosus, antiphospholipid antibody syndrome

HUS or TTP, systemic lupus erythematosus (SLE) and antiphospholipid antibody syndrome may coexist in the same patient. HUS/TTP has been reported in 2–3%[124] and 8.4%[125] of SLE patients, with over 50 cases to date. It may manifest at any age, either before or years after SLE has been diagnosed.[126] The etiology is unclear. Autoantibodies to ADAMTS13, platelets and the platelet glycoprotein CD36 have been described in patients with SLE and it has been postulated that these incite endothelial injury and trigger the release of ultralarge vWF multimers, culminating in TMA.[124] Immunosuppressive and cytotoxic drugs have been used, in conjunction with plasmapheresis, to suppress production and increase clearance of these antibodies, with most patients achieving remission of microangiopathic symptoms. Some respond well to plasmapheresis alone.[124]

HUS/TTP is a rare complication of antiphospholipid antibody syndrome and sometimes its presenting manifestation.[127] The main clinical consequences are severe hypertension, variable degrees of proteinuria, renal impairment, and cortical atrophy. Interestingly, in two patients severe ADAMTS13 deficiency was found due to autoantibodies.[128] Antiphospholipid antibodies have been suggested to increase the risk of TMA in SLE as lupus anticoagulant or anticardiolipin antibodies are found in the majority of SLE patients who develop HUS/TTP.[127]

Glomerular capillary thrombosis is an additional vascular lesion that occurs in SLE, mainly in patients with antiphospholipid antibodies, and leads to glomerular sclerosis and renal insufficiency.[129] Steroids and plasma exchange alone or in combination, are the most common treatment. A meta-analysis showed that where steroids alone were the first treatment used, the clinical status and laboratory abnormalities worsened, whereas recovery occurred in 73% of episodes treated with plasma exchange.[127]

Glomerular disorders

A small number of cases develop HUS superimposed on different forms of glomerular diseases. These are often children who are nephrotic at presentation[130–134] although adults are also described.[135] HUS and membranoproliferative glomerulonephritis may coincide. Both are associated with alternative pathway complement activation and in a few cases where mutations affecting complement FH have been identified the classification would be better described as described in the section on Genetic disorders of complement regulation.

Familial/genetic disorders not included: Part 1

There are current familial cases extensively investigated for the causes advanced in part 1 of the proposed classification with negative results, indicating that other inherited risk factors await recognition.

Unclassified

Presently no cause or clinical association is found in the majority of childhood patients with non-diarrheal HUS, or in adults with HUS/TTP. Mostly these cases have not been comprehensively investigated. However, an undetermined proportion remains unclassified after full evaluation of complement regulation and ADAMTS13 activity.

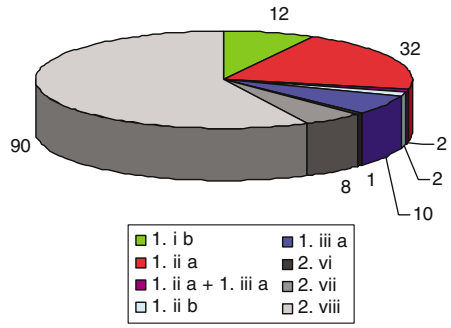

Figure 1 | Sub-group diagnosis in 167 children with non-diarrheal, VTEC negative HUS entered into the registry of the European Pediatric Research Group. Part 1 subgroups are colored sections, part 2 are gray. Of the 34 with mutations in complement regulators, 22 involved FH, six FI, and six MCP. Two further cases had acquired anti-FH antibodies. Abnormality in ADAMTS13 protease and or gene was found in 12 cases, two of whom also had a complement FH mutation. HUS attributable to pneumococcal sepsis occurred in 12 children. There were eight cases, four sibling pairs, in whom the etiology was unknown but the disorder was clearly familial. In one case HUS complicated existing mesangioproliferative glomerulonephritis. In 22 of the unclassified cases complement disregulation was suspected because of relapsing disease and low C3 concentration, or abnormal Western blot profile of complement FH.

TESTING THE PROPOSED CLASSIFICATION

Figure 1 shows the diagnoses in 167 children with HUS in the European Paediatric Registry. Cases with diarrhea or VTEC infection (95% of all pediatric HUS) are excluded. In summary, 58 cases could be allocated to a level 1 diagnostic category, 35% of the total. Few of the 90 cases with no known causation have had complete investigation for complement dysregulation or ADAMTS13 activity.

DISCUSSION

Diagnostic terms need precision to be useful. HUS and TTP describe clinical presentations in the *absence* of knowledge about the cause, and even then may confuse. For example, there is no advantage in relabelling a case of VTEC-induced 'HUS' as 'TTP' because of neurological events that may affect over 10% of children. 'Atypical HUS,' often used to indicate presentation without diarrhea, is clearly a misnomer if it refers to a *typical* example of a known subgroup, for example HUS induced by pneumococcal sepsis. 'Intrinsic' and 'extrinsic' HUS/TTP is a laudable attempt to separate patients with host factors that promote recurrence and poor prognosis from those whose disease is the result of a single environmental insult.[136] However, this bipolar concept does not address any interplay between genetic and environmental factors. TMA is a pathological term and should be confined to this context.

Can the term TTP be adapted to indicate etiology and pathogenesis? Given that severe deficiency of ADAMTS13, has been identified mostly in those described clinically as TTP, while normal activity is usually found in VTEC-induced-, quinine-induced-, and bone marrow transplant-associated HUS/TTP, this has been proposed. However, separation of TTP from HUS on the primary role of ADAMTS13 activity is not without its critics.[137] A wide range of ADAMTS 13 activity can be found in various physiological and disease states, and a profound reduction has been shown in sepsis.[138] In an unselected series of 111 adult cases, severe ADAMTS13 deficiency provided 89% sensitivity and 91% specificity for what was clinically diagnosed as TTP.[122] In another unselected series of 142 adults, only 18 cases had profound deficiency, and this finding did not distinguish them from the 32 with 'idiopathic HUS/TTP' without decreased activity.[123] Our proposal allows etiological and clinical descriptions to operate independently.

Some patients have two different etiologies, for example both VTEC-induced HUS and loss of ADAMTS13 activity,[139] decreased ADAMTS13 plus a FH mutation [140] and a FH mutation plus cobalamine deficiency.[141] For any individual it may be necessary but not in itself sufficient that there is a mutation in FH, or a VTEC infection. There are good reasons to consider 'two-hit' or catastrophic models for disease expression. A mutated complement regulator may be a predisposition, VTEC infection a trigger. The proposed classification accommodates this way of thinking about causation. Dual causation will be missed if clinicians fail to explore further after a seemingly satisfactory diagnosis has been reached.

In any case of HUS/TTP, the history and the clinical pattern is important and may indicate an appropriate subgroup and therapy. Nevertheless, we propose that there should be comprehensive investigation of all cases with the only exception of a first episode of VTEC-positive, diarrhea-associated HUS in a child in whom there is no family history of HUS or TTP.

The difficulty in reaching an etiological diagnosis is considerable. Relevant tests have hitherto been available only through research laboratories. There is a pressing need for laboratory services to be available for the assessment of ADAMTS13 activity, and the genetic and functional investigation of complement regulation. To assist clinicians the European Paediatric Research Group for HUS maintains a website to indicate those laboratories that are able to assist (http://espn.uwcm.ac.uk/guidelines.htm).

An agreed classification is necessary if disease registries are to make progress in correlating clinical and etiological aspects. Without this, advice about treatment often lacks evidence,[142] and treatment trials are difficult to design. We consider that the present classification will assist with this process. However, as no etiological diagnosis can be found in some thoroughly investigated cases, it is likely that additional diagnostic subgroups are still out there to be discovered. Our proposal is therefore an interim one destined for further development.

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