Atypical HUS is more common than TTP and STEC-HUS: results from the "Cross-sectional evaluation of clinical symptoms and epidemiologic parameters in patients with TMA, differentiated by laboratory parameters" study (CESAR)

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INTRODUCTION

Atypical haemolytic uraemic syndrome (aHUS), thrombotic thrombocytopenic purpura (TTP) and HUS caused by Shiga toxin-producing *Escherichia coli* (STEC-HUS) are serious yet rare diseases of thrombotic microangiopathy (TMA). These diseases have similar clinical presentations with consumption of thrombocytes, microangiopathic haemolytic anaemia (MAHA) and end-organ damage. TTP is caused by a severe deficiency of von Willebrand factor-cleaving protease ADAMTS13 (***a d**isintegrin **a**nd **m**etalloproteinase with a **t**hrombo**s**pondin type 1 motif, member 13*) and diagnosed by evaluation of the ADAMTS13-activity in citrate plasma. STEC-HUS is diagnosed by detection of Shiga-toxin in patient stool samples. aHUS is caused by a defect in complement regulation. In aHUS there is often an endogenous predisposition combined with an external complement-amplifying condition (CAC).[1] As there is no definitive diagnostic test for aHUS, it remains a diagnosis of exclusion.

Epidemiological data on TTP and aHUS in Germany are lacking. Furthermore, the relative incidence (RI) of aHUS, TTP, and STEC-HUS, in patients (pts) presenting with TMA is not known.

OBJECTIVE

To evaluate the RI of aHUS, TTP, and STEC-HUS, in patients presenting with TMA in Germany.

METHODS

- CESAR is a prospective, multicentre, cross-sectional, non-interventional epidemiological investigation of the RI of aHUS, TTP and STEC-HUS in clinically suspected cases of TMA.
- The study was approved by the ethics committee of the Bayerische Landesärztekammer. All patients signed informed consent prior to inclusion.
- Blood and stool samples were analysed in reference laboratories for detection of STEC and analysis of ADAMTS13-activity.

Subjects

Inclusion criteria:

Each patient had to meet all of the following criteria:

- MAHA (demonstrated e.g. by the presence of schistocytes on microscopy of the blood film, increased LDH, decreased haemoglobin, decreased haptoglobin).
- Consumption of thrombocytes: platelet count <150 x10^3/µL or decrease of platelet count >25% within one week.
- One of the following:
  - Neurological symptoms, e.g. confusion, cerebral changes, convulsions/ spasms, dysarthria, dysphasia, aphasia, impaired consciousness or others according to clinical assessment of investigator.
  - Renal impairment: e.g. increased creatinine, decreased estimated glomerular filtration rate (eGFR), increased blood pressure, or abnormal urinalysis (e.g. proteinuria).
  - Gastrointestinal symptoms: e.g. diarrhoea with or without blood, nausea or vomiting, abdominal pain, or gastroenteritis.

Exclusion criteria:

- Plasma intervention prior to blood sampling for ADAMTS13 analysis.

STEC testing

- PCR was performed on all stool samples following 18–24 hour incubation in an enrichment broth.
- Samples were analysed for Shiga toxin 1 (stx1), Shiga toxin 2 (stx2), intimin (eaeA) and enterohemorrhagic *Escherichia coli* (EHEC) strain O157 genes.
- PCR results were verified by immunomagnetic separation or next generation sequencing analysis.

ADAMTS13-activity

- Plasma ADAMTS13-activity was assessed using the commercially available Technozyme ELISA (Technoclone, Vienna, Austria).
  - The reference range of ADAMTS13-activity is 50–110% of normal.
  - Assay sensitivity for ADAMTS13-activity in plasma was 0.5%.

Diagnoses

- Treating clinicians diagnosed patients as either aHUS, TTP, STEC-HUS or "other" based on results of ADAMTS13-activity, STEC-test, and other clinically relevant diagnostic information.
- Diagnosis could be revised until the end of the study if additional information became available.
- Within the aHUS group, details of potential CAC were also recorded.
- For patients with "other" diagnosis, the diagnoses were entered as free text.

Data analysis

- Analyses were carried out on intention-to-diagnose basis.
- Descriptive statistics were used.
- Patients were grouped by age at enrolment (paediatric <18 years; adult ≥18 years).
- The primary endpoint was the RI of aHUS, TTP, STEC-HUS and "other".

RESULTS

- From 25th April 2014 to 31st March 2017, 232 patients were enrolled in 22 centres across Germany.

Patient characteristics

Demographic and clinical data of the patients according to differential diagnosis are shown in Tables 1 and 2.

- Overall, mean age at enrolment was 53.3 years, 219/232 (94%) patients were adults, and 125/232 (54%) were female.
- Patients with >1 extrarenal organ involved were common, irrespective of the differential diagnosis.
  - Gastrointestinal symptoms were the most prevalent extrarenal complication in patients with STEC-HUS (86%) and aHUS (50%). All four paediatric patients with aHUS had gastrointestinal symptoms. In two of them infectious diarrhoea, other than STEC, was identified as the CAC.
  - Neurological symptoms were the most common complication for patients with TTP (58%) and were also common in patients with aHUS (40%).
- Patients with TTP had a lower platelet count (median platelet count 26.0 x10^3/µL) than patients with STEC-HUS (53.0 x10^3/µL) or aHUS (98.5 x10^3/µL).

Table 1. Demographic and clinical characteristics of adult patients (n=219).

Characteristic	aHUS (n=137)	TTP (n=31)	STEC-HUS (n=5)	Other (n=46)
Age at enrolment (years), mean (SD)	56.9 (18.6)	50.5 (16.7)	58.4 (24.0)	57.5 (20.7)
Gender, female, n (%)	79 (58)	21 (68)	3 (60)	17 (37)
Presenting symptoms, n (%)				
Renal	127 (93)	22 (71)	5 (100)	43 (93)
Gastrointestinal	67 (49)	11 (35)	4 (80)	23 (50)
Neurological	56 (41)	18 (58)	2 (40)	10 (22)
Cardiovascular	51 (37)	7 (23)	2 (40)	4 (9)
Pulmonary	48 (35)	4 (13)	0 (0)	5 (11)
Other	44 (32)	10 (32)	2 (40)	21 (46)
Platelets (10^3/µL), median (IQR)	99.5 (47.0–124.5)	26.0 (12.0–57.0)	37.0 (34.0–110.0)	61.0 (29.0–83.0)
Haemoglobin reduced below LLN, n (%)	130 (95)	25 (81)	5 (100)	40 (87)
Serum creatinine (mg/dL), median (IQR)	2.1 (1.3–3.7)	1.1 (0.9–1.4)	3.2 (3.1–5.3)	2.2 (1.3–3.8)
Bilirubin (mg/dL), median (IQR)	1.0 (0.6–2.0)	2.1 (1.9–2.7)	2.1 (0.6–2.3)	1.4 (0.7–3.2)

aHUS, atypical haemolytic uraemic syndrome; IQR, interquartile range; LLN, lower limit of normal; SD, standard deviation; STEC-HUS, Shiga toxin-producing *Escherichia coli*-HUS; TTP, thrombotic thrombocytopenic purpura.

Table 2. Demographic and clinical characteristics of paediatric patients (n=13).

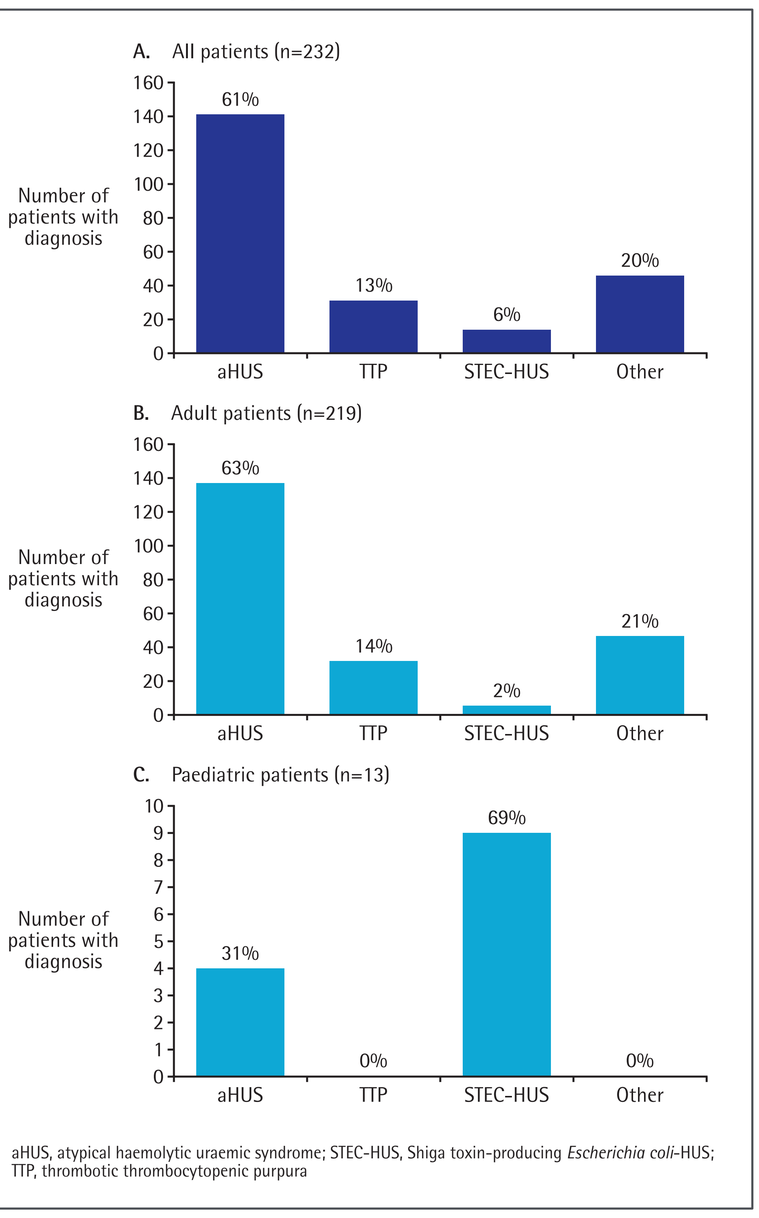
Characteristic	aHUS (n=4)	STEC-HUS (n=9)
Age at enrolment (years), mean (SD)	4.0 (2.0)	5.3 (5.8)
Female, n (%)	0 (0)	5 (56)
Presenting symptoms, n (%)		
Renal	4 (100)	9 (100)
Gastrointestinal	4 (100)	8 (89)
Neurological	1 (25)	2 (22)
Cardiovascular	0 (0)	0 (0)
Pulmonary	0 (0)	0 (0)
Other	0 (0)	0 (0)
Platelets (10^3/µL), median (IQR)	54.5 (17.8–95.3)	58.0 (40.0–88.0)
Haemoglobin reduced below LLN, n (%)	4 (100)	8 (89)
Serum creatinine (mg/dL), median (IQR)	1.5 (0.9–4.2)	5.0 (3.4–5.3)

aHUS, atypical haemolytic uraemic syndrome; IQR, interquartile range; LLN, lower limit of normal; SD, standard deviation; STEC-HUS, Shiga toxin-producing *Escherichia coli*-HUS; TTP, thrombotic thrombocytopenic purpura.

Differential diagnosis

- aHUS was the most common form of TMA observed (Figure 1).
- The RI of aHUS was 61% overall; 63% in adult and 31% in paediatric patients respectively.
- STEC-HUS was infrequently observed in adult patients of our cohort, 5/219 (2%).
- Paediatric patients were either diagnosed with aHUS or STEC-HUS; no TTP or "other" diagnosis was recorded for this small group (n=13).
- One paediatric and one adult patient with confirmed STEC-HUS did not present with gastrointestinal symptoms.

Figure 1. Distribution of TMA diagnoses in A. all patients, B. adult patients and C. paediatric patients.



aHUS, atypical haemolytic uraemic syndrome; STEC-HUS, Shiga toxin-producing *Escherichia coli*-HUS; TTP, thrombotic thrombocytopenic purpura

- Overall, 46 patients were diagnosed as "other" (Table 3).
  - Septicaemia was the most common single "other" diagnosis (n=8).
  - 11 patients were diagnosed with secondary TMA, of which solid tumour was the most common cause.

Table 3. All 'other' investigator-made diagnoses (n=46).

Other diagnosis	n (%)
Secondary TMA:	11 (24)
Solid tumour	5 (11)
Drugs	2 (4)
Infection	2 (4)
Malignant hypertension	1 (2)
Other	1 (2)
Septicaemia	8 (17)
Haematological disease/bone marrow failure	4 (9)
Liver failure	4 (9)
Infection	3 (7)
ITP	3 (7)
Unspecified TMA	3 (7)
Haemolysis mechanical valve	2 (4)
Renal disease	2 (4)
HELLP	1 (2)
Unclear pain syndrome	1 (2)
Thromboembolic disease (secondary to dilated carotid sinus)	1 (2)
Intermittent leukopenia, anaemia and thrombocytopenia	1 (2)
Unclear haemolysis and thrombocytopenia	1 (2)
Allergy to dialysis filter	1 (2)

HELLP, haemolysis elevated liver enzymes low platelet count; ITP, idiopathic thrombocytopenic purpura.

Prevalence of complement-amplifying conditions in patients with aHUS

- Many patients with aHUS had a CAC (Table 4).
  - Some patients had more than one CAC.
  - Calcineurin inhibitors, prior organ transplantation and airway infections were the most common.

Table 4. Complement-amplifying conditions identified in all patients diagnosed with aHUS.

Complement-amplifying condition	n (%)
Calcineurin inhibitors	30 (21)
Organ transplantation	25 (18)
Airway infections	17 (12)
Malignant arterial hypertension	8 (6)
Pregnancy	7 (5)
Gemcitabine	6 (4)
Vasculitides	4 (3)
Organ rejection	4 (3)
Systemic lupus erythematosus	3 (2)
IgA nephropathy	3 (2)
Infectious diarrhoea (other than STEC)	3 (2)
Vaccination	3 (2)
Inflammatory bowel disease	2 (1)
C3 glomerulopathy	2 (1)
Hepatitis C, D	2 (1)
mTOR inhibitors	2 (1)
Varicella-zoster virus	1 (1)
Oral contraceptives	1 (1)
No complement-amplifying condition recorded	37 (26)

DISCUSSION

This is the first study to prospectively evaluate the relative frequency of aHUS, TTP and STEC-HUS in Germany.

- aHUS was the most common single diagnosis in adult patients presenting with MAHA, thrombocytopenia and end-organ damage.
- In children, STEC-HUS was more common than aHUS and TTP was not diagnosed. However, due to the small number of children recruited in this study no firm conclusions can be drawn. TTP has been reported in children and adolescents in the literature.[2,3]
- There was a strong overlap in the clinical presentation of patients. Renal, neurological and gastrointestinal symptoms were found at a high frequency in all diagnosed conditions. This illustrates the difficulty of differentiating TMAs based on clinical symptoms alone.

The classification of aHUS is currently a matter of debate.[4,5] Our diagnoses were made based on the investigator's clinical judgement after retrieval of the results of ADAMTS13-activity and STEC testing, and consideration of the clinical picture. The study illustrates the overlap between aHUS with CAC and other diagnoses highlighting the variability of criteria used to differentially diagnose individual patients even in experienced centres.

Our study has several limitations. Paediatric centres were under-represented, limiting the significance of findings in this group. Adult patients were mainly enrolled from nephrology departments. Therefore, we cannot exclude that the distribution of diagnoses could be different at haematology centres or other departments including obstetrics. Also, inclusion required the presence of MAHA and thrombocyte consumption, therefore, we may have missed patients with incomplete presentation of aHUS.[6] The study was not designed to assess the absolute incidence of the three TMA diseases, we evaluated the rate or "relative incidence" of the three diseases. However, the relative incidence is relevant for the treating physician, as it informs on the pre-test probability of the respective disease when managing a specific patient.

CONCLUSIONS

- In this cohort, aHUS was the most common single diagnosis in adult patients presenting with MAHA, thrombocytopenia and end-organ damage.
- Other less frequent but important differential diagnoses including TTP should be rapidly ruled out through specific diagnostic tests.

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