BMC
Nephrology

RESEARCH ARTICLE   Open Access

Thrombocytopenia is not mandatory to diagnose haemolytic and uremic syndrome

Marion Sallée[1†], Khalil Ismail[1†], Fadi Fakhouri[2], Henri Vacher-Coponat[1], Julie Moussi-Francés[1],
Véronique Frémaux-Bacchi[3] and Stéphane Burtey[1*]

Abstract

Background: Hemolytic and uremic syndrome (HUS) diagnosis involves association of non immune hemolytic anemia, thrombocytopenia, and renal failure. HUS without thrombocytopenia has been observed, we call it partial HUS. Its real frequency and outcome are unknown. The aim of this study was to determine the prevalence of patients with normal platelets count in two HUS cohorts and to compare their outcome to patients with thrombocytopenia.

Methods: We retrospectively identified HUS diagnosis in two different cohorts. The first cohort was from a single center and consisted of all cases of HUS whatever the aetiology, the second was multicentric and consisted of atypical HUS patients. These cohorts were divided into two groups depending on the presence or absence of thrombocytopenia. Clinical and biological data were compared between thrombopenic and non thrombopenic group.

Results: We identified 13% (20/150) of patients with normal platelets count: 10 episodes (18%) of HUS in six patients (14%) in the monocentric cohort and 14 patients (13%) with 17 episodes (12%) in the multicentric cohort of atypical HUS. Groups differed in platelets count and LDH level. In both cohorts, renal outcome was similar to patient presenting with thrombocytopenia.

Conclusion: HUS with normal platelets count is not infrequent. Relative to classical clinical presentation of HUS, partial HUS has similar characteristics and identical poor renal outcome and so must be treated in the same way.

Keywords: Hemolytic and uremic syndrome, Thrombocytopenia, Complement

Background

Thrombotic microangiopathy (TMA) is defined as a typical histology characterized by intimal proliferation and/or endothelial swelling with luminal fibrin deposition in arterial or capillary beds. In the kidney, mesangiolysis, red-cell fragments and double contours of capillary basement can also be found in TMA [1]. TMA is typically associated with non-immune mechanical haemolytic anaemia, thrombocytopenia and organ dysfunction.

Two major syndromes can be distinguished: the haemolytic uremic syndrome (HUS) and the thrombotic thrombocytopenic purpura (TTP) depending on, in general speaking, the main organ function altered, kidney in HUS and brain in TTP. Differences in pathogenicity are observed. TTP is

associated with a defective function of the von Willebrand factor cleaving protease (ADAMTS13) [2,3]. HUS typically results from food poisoning with enterohemorrhagic strains of *Escherichia coli* or other bacteria producing a shiga-like toxin (STEC-HUS). Mutations in *CFH, CFI, CFB, MCP,* and *C3* predispose to atypical HUS (aHUS) [4-10]. Secondary causes of HUS, due to variety of causes (such as drugs, malignancy, systemic disease…) occur not only in adults but also in childhood.

Partial HUS (normal platelet count) is not well characterized; we believed that this can lead to misdiagnosis of HUS and as a result, undertreated patients or at least delayed therapy. This delay could reduce survival.

If partial HUS is a known entity, its real frequency is unknown. In literature, few studies address this fact [11,12]. An occurrence between 0% and 44% is reported. Therefore we conducted a study to describe the real prevalence of subjects with normal platelets count in a

* Correspondence: stephaneb@ap-hm.fr
†Equal contributors
[1]APHM, Hopital de la Conception, centre de néphrologie et transplantation rénale, Aix-Marseille Univ, Marseille 13005, France
Full list of author information is available at the end of the article

single nephrology centre adult cohort of HUS, reflecting the whole spectrum of HUS. In order to confirm prevalence of this entity, we also analyzed a multicentre French cohort of adults with aHUS.

Methods

We conducted a retrospective study in the nephrology centre at Hôpital La Conception (AP-HM, Marseille, France). Using a computerized database, we identified all patients with a HUS diagnosis admitted between January 2000 and June 2010. All patients' medical records were reviewed. Relevant clinical and biological data were collected. Children were excluded.

Partial HUS was suspected by the association of

- a mechanical (with schizocytes on blood smear), non immune (negative coombs test) haemolytic anaemia (haemoglobin under 12 g/dl, haptoglobin under 0,4 g/l and lactate deshydrogenase (LDH) >250 UI/L)
- and acute renal failure (blood creatinine >120 μmol/l).

To confirm the diagnosis of partial HUS, patients were required to have either biopsy proven TMA, and/or mutation in genes coding for proteins involved in the complement alternative pathway, and/or exposition to a drug associated with HUS like gemtacibine and/or recurrence of partial HUS associated with response to plasmatherapy.

Complete HUS was diagnosed by the association of a mechanical, non immune haemolytic anaemia, thrombocytopenia (platelets count below 150 G/L) and acute renal failure.

TTP was excluded by ADAMTS 13 activity above 20%.

We retrospectively compared the group of patients with thrombocytopenia with those with normal platelets count at diagnosis. Treatments before hospital admission, clinical and biological data on admission, treatments administered, clinical outcome, renal outcome and the number of relapses were collected. In cases of relapses, we noted whether the diagnostic criteria were the same.

Platelets counts were taken on the admission. However partial HUS group was defined by the fact that during all the disease, platelets count never decrease below 150 G/L.

Data from the thrombocytopenic group and from the partial HUS group were analyzed further by Fisher's exact tests and Mann–Whitney test (two tailed) analyses.

To confirm our main result, we used a multicentre French database to identify adult with aHUS referred between 2000 and 2008 to the laboratory of immunology at Hôpital Européen Georges Pompidou (Paris, France), the French reference centre for the evaluation of complement disorders in human diseases. Diagnostic criteria used were the same as those used for the first cohort.

Children were excluded. Nine patients included in the first cohort were excluded. Clinical and biological data were collected by the physicians who referred the patients. So, while the Marseilles cohort included all coming HUS patients regardless of the cause, the multicentre French cohort was most restrictive, limited to aHUS.

This study is in compliance with the Helsinki Declaration. We did not need the approval of an ethic committee to access and use the data. The access to hospital files was given by the head of the department of nephrology in Marseilles and the head of the department of immunology for the French cohort.

Results

In the Marseille database, we identified 43 patients, presenting 56 episodes of HUS, over the course of ten years. Six of the 43 patients (14%) had a total of 10 episodes (10/56, 18%) of partial HUS.

Patients' characteristics are summarized in Table 1. Patients were young (median age: 45 years [25–64]) with a majority of females (60%). Aetiologies of HUS were those commonly described in HUS such as toxic causes, mutations in proteins involved in alternative pathway of the complement, and post infectious diseases.

All patients exhibited an acute renal failure (median plasma creatinine level: 3.4 mg/dl [2.4-5.5]) and mechanical, non immune haemolytic anaemia (median Hb: 7.6 g/dl [7.1-8.9], median LDH: 1012 UI/L [607–202], and haptoglobin was not measurable). No difference was observed for creatinine level, haemoglobin level and proteinuria between the thrombocytopenic and the partial HUS groups, while LDH levels and schizocytes count were higher in the thrombocytopenic group.

Relapses were described in 7 patients (7/37, 19%) in the thrombocytopenic group and in 3 patients (3/6, 50%) in the partial HUS group. Platelets count (<150 G/L or ≥ 150 G/L) during relapses were similar to that at first episode.

Plasmatherapy were used for both groups with no differences between number of treated patient and number of plasmatherapy session for an episode.

Table 2 summarized patients' characteristics for the French cohort. We identified 107 patients presenting 145 episodes of HUS. Fourteen of the 107 patients (13%) presented 17 episodes (17/145, 12%) of partial aHUS. Aetiologies in this cohort represent a large panel of mutations in proteins implicated in complement alternative pathway or a familial history of HUS. As in the Marseille cohort, the LDH levels were significantly higher in the thrombocytopenic aHUS group compared to the partial aHUS group. For all other variables studied, no difference was observed.

To assess the severity of the episode, we evaluated the renal outcome one month after the onset of HUS. Because baseline biological data of the two cohorts seemed

Table 1 Data from the Marseille cohort

	All patients	Patients with normal platelets count	Patients with thrombocytopenia	p
Number of patients	43	6	37	
Number of episodes	56	10	46	
Number of relapsing patients	10	3	7	0.88
Sex (%male)	40%	17%	43%	0.40
Age (years)	45 [25–64]	40 [32–50]	44 [23–66]	0.78
Biopsy proven TMA	10 (23%)	3 (50%)	7 (19%)	0.38
Etiology identification	**24 (56%)**	**3 (50%)**	**21 (57%)**	
Identified mutation	6	**2**	4	
Anti-factor H Autoantibody	1		1	
Toxic	5	**1**	4	
Cancer associated	2		2	
Graft associated	2		1	
Autoimmune disease	3		3	
Shiga like toxin	4		4	
Post infectious disease	2		2	
Potential explanation	**10 (23%)**	**2 (33%)**	**8 (22.5%)**	
Familial form	2		2	
Recurrent form	4	2	2	
Associated with low C3	4		4	
Idiopathic	**9 (21%)**	**1 (17%)**	**8 (22.5%)**	
Pregnancy associated HUS	3 (11,5%)	2 (40%)	1 (5%)	0.151
*Platelets (G/L)**	66 [55–108]	234 [183–265]	53 [27–88]	< 0.001
Hb (g/dl)	7.6 [7.1-8.9]	8 [7.2-9.8]	7.7 [7.1-8.8]	0.38
LDH (UI/L)*	1012 [607–202]	612 [400–705]	1074 [659–2192]	0.012
Haptoglobin	0 [0–0]	0 [0–0,28]	0 [0–0]	0.064
*Schizocytes %**	2.2 [1.5-3.8]	0.8 [0–2]	3 [1.5-4.4]	< 0.001
Creatinine (mg/dl)	3.4 [2.4-5.5]	2.5 [1.4-5.3]	3.5 [2.5-5.6]	0.76
Proteinuria (g/24h)	2.2 [1.1 – 3.6]	1.5 [0.8 – 2.3]	2.32 [1.2 – 4]	0.15
HD at the diagnosis	12 (21%)	2 (20%)	10 (22%)	0.68
HD during the episode	34 (61%)	5 (50%)	29 (63%)	0,6
Death (%)	1 (2%)	1 (17%)	0 (0%)	0,6
Plasmatherapy				
Number of episodes treated	52 (93%)	8 (80%)	44 (96%)	0.88
Number of plasmatherapy sessions	15 [12–18]	15 [6–16.5]	14 [12–20]	

For Numeric value, data are express in Median value [25%-75%], for categories, data are express in number of observation (%). Test used, Mann Whitney test (quantitative data) and Fisher's test for proportion and rate. *Note,* Conversion factors for units, serum creatinine in mg/dL to mol/L, ×88.4; serum urea nitrogen in mg/dL to mmol/L.

quite similar and in order to increase the power of the study, we pooled the two cohorts. Table 3 summarizes the outcome. 34 patients over 150 were lost-to follow up. HD was required at the acute phase in 106/150 (71%) patients. 65/150 patients (43%) remained HD-dependant. 20/150 patients (13.6%) had persistent renal failure, and only 24/150 patients (16%) had complete recovery of their renal function. No statistical difference could be highlighted between the two groups.

Discussion

We report here an occurrence of 13% (20/150) of partial HUS patients defined by normal platelets count at diagnosis in two independent French cohorts. Thrombocytopenia was still absent in relapses when it was absent during the first episode. This suggests that the occurrence of partial HUS is frequent. No specific cause of partial HUS was associated with lack of thrombocytopenia.

Table 2 Data from the multicentric French cohort

	All patients	Patients with normal platelets count	Patients with thrombocytopenia	P
Number of patients	107	14	93	
Number of episodes	145	17	128	
Number of relapsing patients	18 (20%)	2 (14%)	19 (20%)	0.88
Sex (%male)	29 (27%)	5 (36%)	24 (26%)	0.39
Age (years)	32 [22 – 45]	32 [22 – 45]	33 [23 – 42]	0.78
Biopsy proven TMA	72 (67%)	13 (93%)	59 (63%)	
identification of mutation	**75 (70%)**	10 (71%)	65 (70%)	0.95
CFH	40	6	34	
CFI	17	3	14	
MCP	10	1	9	
C3	11	1	10	
CFB			2	
Anti-factor H Autoantibody			2	
Potential explanation	**2 (13.3%)**	**1 (14.3%)**	**11 (12%)**	
Familial form		1	10	
Low C3	2	0	1	
Idiopathic	**21 (23.3%)**	**3 (21.4%)**	**19 (25%)**	
Pregnancy associated HUS	19 (18%)	1 (7%)	17 (18%)	0.151
Platelets (G/L)*	79 [48-117]	214 [186 - 242]	68 [46-100]	< 0.001
Hb (g/dl)	7.2 [6,6 - 9]	8.6 [6.7-10]	7.2 [6.6 - 8.8]	0.16
LDH* (UI/L)	1688 [951-2930]	664 [498-1914]	1711 [999-1953]	0.017
Creatinine (mg/dl)	7.3 [4.1 - 11.3]	4.3 [2.6 - 12]	7.6 [4.6 - 11.3]	0.27
HD at the diagnosis	13 (12%)	1 (7%)	12 (13%)	0.68
HD during the episode	72 (80%)	10 (71%)	62 (81.6%)	0.6
Death Number/%	6 (6.7%)	1 (7.1%)	5 (6.6%)	0.9

For Numeric value, data are express in Median value [25%-75%], for categories, data are express in number of observation (%). Test used, Mann Whitney test (quantitative data) and Fisher's test for proportion and rate. Note, Conversion factors for units, serum creatinine in mg/dL to mol/L, ×88.4; serum urea nitrogen in mg/dL to mmol/L.

Normal platelets count in TMA has already been described in previous study. Veyradier et al. [12] reported an occurrence of 40%. However, in this study, patients included had either HUS or TTP, with a high proportion of secondary TMA. In others clinical reports, Sellier-leclerc et al. reported an occurrence of 15% in a cohort of aHUS with complement mutation [13] and Fakhouri et al. [14] described 3 over 21 aHUS with normal platelets count in pregnancy associated HUS. In those articles details are not mentioned and little is known about platelets count evolution but the occurrence of normal platelets count seems similar to what we noticed. Recently, De Serre et al. [11] described an occurrence of 44% of patient with TMA and normal

Table 3 Renal outcome three months after the last episode

	Total	Patients with normal platelets count	Patients with thrombocytopenia	p
Number of patient	150	20	130	
Death	7 (4.7%)	2 (10%)	5 (4%)	0.55
ESRD leading to chronic haemodialysis	65 (43%)	9 (45%)	56 (43%)	0.28
CKD	20 (13.6%)	4 (20%)	16 (12%)	0.33
Recovery	24 (16%)	3 (15%)	21 (16%)	0.75
Lost	34 (22%)	2 (10%)	32 (25%)	0.21

CKD, chronic kidney injury estimated GFR in ml/min/1.73 m² (MDRD simplified) <60 ml/min/1.73 m². Fisher's test analysis.
ESRD, End stage renal disease.

platelets count in a cohort of histological TMA. Nevertheless, some of the patients did not present with haemolytic anaemia. Consequently, we assumed that some of their description included localized TMA. In our study, patients with partial HUS reflect the whole TMA spectrum in a nephrology centre. Our study put in light that biopsy is difficult to manage in the course of HUS. Consequently, histological diagnosis of HUS is often difficult to confirm. One limitation of our study is in some patients with partial HUS, a kidney biopsy lacks to absolutely confirm the diagnosis of renal TMA. We have only indirect clinical evidence by the presence of a mutation in the genes coding for complement alternative pathway and/or response to plasmatherapy, in favour of the diagnosis of HUS.

Nevertheless, this study underlines a similar renal outcome of HUS whatever the platelets count, even if haemolysis is more severe in cases with thrombocytopenia. This similar outcome is not explained by the fact that in normal platelets count, patient did not experiment plasmatherapy. Renal outcome impacts on the prognosis of HUS. A poor outcome, leading to irreversible kidney injury or death, is described in both groups. This confirms data from De Serres et al. [11]. They described a poor renal outcome and a worse survival in the group with normal platelets count compared to the thrombocytopenic group. The authors argued that a worst outcome may be related to delay in patient care. Our patients with partial HUS had a median delay to diagnosis of 11 days. This can explain the similar outcome in our two groups. We believed that normal platelets count can delay HUS diagnosis and that kidney biopsy is the key to confirm the diagnosis. Thus we recommend performing renal biopsy if it is possible as soon as possible in patient with haemolytic anaemia and kidney impairment. It is also critical to investigate the alternative pathway of complement by measure of the complement protein and research of mutations in the genes controlling this pathway. The diagnosis could elicit the initiation of the treatment of HUS. Our data provide a strong argument that patient with partial HUS would benefit from the same therapy as for thrombocytopenic HUS.

This study questions whether platelets count is the best criterion to evaluate response to treatment in HUS. Indeed, TMA can persist even when thrombocytopenia has resolved. Improvement of kidney function or other organs involvement like brain or heart could be retained as real response criterion in HUS[15]. The LDH levels cannot be used alone as it has already been reported as a less important biomarker [16,17]. More accurate biomarkers of HUS activity are yet to be found to optimise patients' care.

Conclusion
Thrombocytopenia is not necessary to evoke HUS diagnosis. The association of mechanical haemolytic anaemia and renal injury in the absence of thrombocytopenia should lead to perform a renal biopsy in order to diagnose partial HUS. Normal platelets count is not associated with favourable renal prognosis in HUS.

Authors' contributions
MS and KI collected and analyzed the data of the Marseille's cohort and write the first draft of the manuscript, HVC and JMF are involved in the care of the patients of the Marseille's cohort, FF analyzed and maintained the data of the French cohort, VFB provided the mutation screening of complement genes and maintenance of the data of the French cohort, SB designed the study, analyzed the whole dataset and write the manuscript. All authors read and approved the final manuscript.

Author details
[1]APHM, Hopital de la Conception, centre de néphrologie et transplantation rénale, Aix-Marseille Univ, Marseille 13005, France. [2]Département de Nephrologie, INSERM UMR643, CHU de Nantes, 1 place Alexis-Ricordeau, Nantes 44000, France. [3]Service d'immunologie biologique, hôpital Européen Georges-Pompidou, Assistance publique–Hôpitaux de Paris, 20-40, rue Leblanc, Paris cedex 15 75908, France.

References
1. Kaplan BS, Meyers KE, Schulman SL: The pathogenesis and treatment of hemolytic uremic syndrome. J Am Soc Nephrol 1998, 9(6):1126–1133.
2. Tsai HM, Lian EC: Antibodies to von willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. N Engl J Med 1998, 339(22):1585–1594.
3. Furlan M, Robles R, Galbusera M, Remuzzi G, Kyrle PA, Brenner B, Krause M, Scharrer I, Aumann V, Mittler U, et al: von willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. N Engl J Med 1998, 339(22):1578–1584.
4. Fremeaux-Bacchi V, Fakhouri F, Roumenina L, Dragon-Durey MA, Loirat C: Atypical hemolytic-uremic syndrome related to abnormalities within the complement system. Rev Med Interne 2011, 32(4):232–240.
5. Noris M, Remuzzi G: Atypical hemolytic-uremic syndrome. N Engl J Med 2009, 361(17):1676–1687.
6. Saunders RE, Abarrategui-Garrido C, Fremeaux-Bacchi V, Goicoechea de Jorge E, Goodship TH, Lopez Trascasa M, Noris M, Ponce Castro IM, Remuzzi G, Rodriguez de Cordoba S, et al: The interactive factor H-atypical hemolytic uremic syndrome mutation database and website, update and integration of membrane cofactor protein and factor I mutations with structural models. Hum Mutat 2007, 28(3):222–234.
7. Fremeaux-Bacchi V, Moulton EA, Kavanagh D, Dragon-Durey MA, Blouin J, Caudy A, Arzouk N, Cleper R, Francois M, Guest G, et al: Genetic and functional analyses of membrane cofactor protein (CD46) mutations in atypical hemolytic uremic syndrome. J Am Soc Nephrol 2006, 17(7):2017–2025.
8. Goicoechea De Jorge E, Harris CL, Esparza-Gordillo J, Carreras L, Arranz EA, Garrido CA, Lopez-Trascasa M, Sanchez-Corral P, Morgan BP, Rodriguez De Cordoba S: Gain-of-function mutations in complement factor B are associated with atypical hemolytic uremic syndrome. Proc Natl Acad Sci U S A 2007, 104(1):240–245.
9. Kavanagh D, Kemp EJ, Mayland E, Winney RJ, Duffield JS, Warwick G, Richards A, Ward R, Goodship JA, Goodship TH: Mutations in complement factor I predispose to development of atypical hemolytic uremic syndrome. J Am Soc Nephrol 2005, 16(7):2150–2155.

10. Kavanagh D, Kemp EJ, Richards A, Burgess RM, Mayland E, Goodship JA, Goodship TH: **Does complement factor B have a role in the pathogenesis of atypical HUS?** *Mol Immunol* 2006, **43**(7):856–859.
11. De Serres SA, Isenring P: **Athrombocytopenic thrombotic microangiopathy, a condition that could be overlooked based on current diagnostic criteria.** *Nephrol Dial Transplant* 2009, **24**(3):1048–1050.
12. Veyradier A, Obert B, Houllier A, Meyer D, Girma JP: **Specific von willebrand factor-cleaving protease in thrombotic microangiopathies, a study of 111 cases.** *Blood* 2001, **98**(6):1765–1772.
13. Sellier-Leclerc AL, Fremeaux-Bacchi V, Dragon-Durey MA, Macher MA, Niaudet P, Guest G, Boudailliez B, Bouissou F, Deschenes G, Gie S, *et al*: **Differential impact of complement mutations on clinical characteristics in atypical hemolytic uremic syndrome.** *J Am Soc Nephrol* 2007, **18**(8):2392–2400.
14. Fakhouri F, Roumenina L, Provot F, Sallee M, Caillard S, Couzi L, Essig M, Ribes D, Dragon-Durey MA, Bridoux F, *et al*: **Pregnancy-associated hemolytic uremic syndrome revisited in the era of complement gene mutations.** *J Am Soc Nephrol* 2010, **21**(5):859–867.
15. Dorresteijn EM, van de Kar NC, Cransberg K: **Eculizumab as rescue therapy for atypical hemolytic uremic syndrome with normal platelet count.** *Pediatric nephrology* 2012, **27**(7):1193–1195.
16. George JN, Terrell DR, Swisher KK, Vesely SK: **Lessons learned from the oklahoma thrombotic thrombocytopenic purpura-hemolytic uremic syndrome registry.** *J Clin Apher* 2008, **23**(4):129–137.
17. Vesely SK, George JN, Lammle B, Studt JD, Alberio L, El-Harake MA, Raskob GE: **ADAMTS13 Activity in thrombotic thrombocytopenic purpura-hemolytic uremic syndrome, relation to presenting features and clinical outcomes in a prospective cohort of 142 patients.** *Blood* 2003, **102**(1):60–68.