10) Yoshihiro Fujimura, Toshiyuki Miyata, "Thrombotic microangiopathy (TMA) with special references to a registry of congenital TMAs in Japan", The 3rd ASEAN Federation of Hematology, October 23-25, 2014, Bangkok, Thailand

11) Toshiyuki Miyata, "Thrombotic thrombocytopenic purpura and ADAMTS13", 2014 Suzhou International Symposium on Basic and Translational Vascular Research, October 11-13, 2014, Suzhou, China

12) Masanori Matsumoto, Toshiyuki Miyata, Yoshihiro Fujimura, Symposium, "Registry of congenital TMAs in Japan", The 8th Congress of Asia Pacific Society on Thrombosis and Haemostasis-2014, October 9-11, 2014, Hanoi, Vietnam

13) Masanori Matsumoto, Toshiyuki Miyata, Yoshihiro Fujimura, Symposium, "Japanese experience of congenital thrombotic microangiopathies", The 11th Congress of the Asian Society for Pediatric Research, April 15-18, 2015, Osaka.

14) 宮田敏行、内田裕美子、大田敏之、浦山耕太郎、吉田瑤子、藤村吉博「非典型溶血性尿毒症症候群患者に見られた diacylglycerol kinase e の遺伝子変異」第 37 回日本血栓止血学会学術集会 2015 年 5 月 21-23 日、甲府市、山梨県

15) 藤井寛、大田敏之、宮田敏行、浦山耕太郎、多田昌弘、古江健樹、今井清香、松原啓太、小野浩明、坂野堯、神野和彦、吉田瑤子、藤村吉博エクリズマブが著効を示した DGKE 遺伝子異常による非典型溶血性尿毒症症候群の一男児例、第 50 回日本小児腎臓病学会学術集会、2015 年 6 月 18-20 日、神戸市、兵庫県

16) Masanori Matsumoto, Ayami Isonishi, Koichi Kokame, Masaki Hayakawa, Hideo Yagi, Toshiyuki Miyata, Yoshihiro Fujimura, "Characteristics and outcomes of patients with Upshaw-Schulman syndrome receiving maintenance hemodialysis due to chronic renal failure", XXV Congress of the International Society on Thrombosis and Haemostasis, June 20-25, 2015, Toronto, Canada.

17) 宮田敏行、加藤秀樹、内田裕美子、吉田瑤子、小亀浩市、福岡利仁、要　伸也、大田敏之、浦山耕太郎、藤永周一郎、櫻谷浩志、喜瀬智郎、渡邉栄三、織田成人、永田裕子、玉井宏史、小松真太郎、前沢浩司、川村尚久、永野幸治、河野智康、松本雅則、藤村吉博、南学正臣、「日本人の非典型溶血性尿毒症症候群患者の遺伝子解析補体系因子と DGKE の遺伝子変異」第 52 回補体シンポジウム、2015 年 8 月 21-22 日、名古屋市、愛知県

18) T. Miyata, X. P. Fan, H. Shirotani-Ikejima, Y. Eura, H. Hirai, S. Honda, J. A. Kremer Hovinga, M. Mansouri Taleghani, A.S. von Krogh, Y. Yoshida, B. Lämmle, Y. Fujimura, "Mutations in complement factors in patients with Upshaw-Schulman syndrome with renal insufficiency" 優秀ポスター発表、第 77 回日本血液学会学術集会、2015 年 10 月 16-18 日、金沢市、石川県

19) 長谷川真弓、西田幸世、前田美和、辻内智美、門池真弓、馬場由美、下村志帆、内池敬男、早川正樹、松本雅則、藤村吉博、「奈良医大付属病院における輸血後感染症検査の実施状況」第 62 回日本輸血細胞治療学会総会、2014 年 5

月 15 日、奈良市

20）松本雅則、シンポジウム「TTP の診断と治療」第 62 回日本輸血細胞治療学会総会、2014 年 5 月 16 日、奈良市

21）石西綾美、松本雅則、藤村吉博、「後天性 TTP の血漿交換療法の影響による ADAMTS13 自己抗体の動態解析」第 62 回日本輸血細胞治療学会総会、2014 年 5 月 16 日、奈良市

22）早川正樹、松本雅則、吉井由美、八木秀男、藤村吉博、「造血幹細胞移植後の肝中心静脈閉塞症の発症と予防的血小板輸血との関連性」第 62 回日本輸血細胞治療学会総会、2014 年 5 月 16 日、奈良市

23）八木秀男、早川正樹、山口尚子、山下慶悟、松本雅則、谷口繁樹、杉本充彦、椿和央、藤村吉博、「重症大動脈弁狭窄症患者の弁置換術前後における VWF 依存性血小板血栓形成の計時的変化の検討」第 36 回日本血栓止血学会学術集会、2014 年 5 月 31 日、大阪市

24）樋口（江浦）由佳、小亀浩市、高蓋寿朗、田中亮二郎、小林光、石田文宏、久永修一、松本雅則、藤村吉博、宮田敏行、「ダイレクトシークエンシング、定量 PCR、次世代シークエンシングを用いた TTP 患者の遺伝子解析」第 36 回日本血栓止血学会学術集会、2014 年 5 月 31 日、大阪市

25）早川正樹、松本雅則、八木秀男、天野逸人、田中晴之、木村弘、藤村吉博「造血幹細胞移植患者における好中球生着と UL- VWFM 出現との関連」第 36 回日本血栓止血学会学術集会、2014 年 5 月 31 日、大阪市

26）松本雅則、早川正樹、石西綾美、吉田瑶子、吉井由美、田中賢治、前田琢磨、宮田茂樹、藤村吉博「維持血液透析患者の血小板減少に対する抗血小板第 4 因子／ヘパリン抗体の関与」第 36 回日本血栓止血学会学術集会、2014 年 5 月 31 日、大阪市

27）小亀浩市、樋口（江浦）由佳、松本雅則、藤村吉博、宮田敏行、「デジタル PCR を用いた ADAMTS13 遺伝子変異のヘテロ接合性の解析」第 36 回日本血栓止血学会学術集会、2014 年 5 月 29-31 日、大阪市

28）松本雅則 特別講演「TTP の診断と治療」第 15 回日本検査血液学会学術集会、2014 年 7 月 20-21 日

29）松本雅則、シンポジウム「腎臓内科領域におけるアフェレシスの未来展望～血栓性微小血管障害症に対する治療法の進歩～」第 35 回日本アフェレシス学会学術大会、2014 年 9 月 28 日、東京都

30）宮川義隆、松本雅則、上田恭典、村田満、阿部貴行、三宅真二、菊池佳代子、岡本真一郎、太田秀一、半田寛、朝倉英策、和田英夫、西尾健治、椿和央、日笠聡、野村昌作、一戸辰夫、藤村吉博、「血栓性血小板減少性紫斑病に対するリツキシマブの第 2 相医師主導治験」第 76 回日本血液学会学術集会、2014 年 10 月 31 日-11 月 2 日、大阪市

31） Hayakawa M, Yagi H, Yamaguchi N, Yamashita K, Hayata Y, Abe T, Taniguchi S, Fujimura Y, Matsumoto M“The changes of von Willebrand factor multimers in patients with aortic stenosis by valve replacement”第 76 回日本血液学会学術集会、2014 年 10 月 31 日-11 月 2 日、大阪市

32）Yagi H, Hayakawa M, Yamaguchi N, Yamashita

K, Taniguchi S, Matsumoto M, Tsubaki K, Fujimura Y."Decreased platelet thrombus size, due to a heightened proteolysis of VWF by ADAMTS13 is quickly restored after valve replacement in aortic stenosis patients."The 56th Annual meeting of American Society of Hematology、2014 年 12 月 7 日、San Francisco/USA

33） Yoshii Y, Matsumoto M, Kurumatani N, Isonishi A, Uemura M, Hori Y, Hayakawa M, Yagi H, Bennett CL, Fujimura Y."Introduction of a quick assay for ADAMTS13 activity improved a survival of acquired TTP patients who received platelet transfusions."The 56th Annual meeting of American Society of Hematology、2014 年 12 月 8 日、San Francisco/USA

34） 早川正樹、藤村吉博、松本雅則「von Willebrand 因子による造血幹細胞移植後 TMA/VOD の病態解析」第 37 回日本造血細胞移植学会総会、2015 年 3 月 6 日、神戸市

35）森山雅人、玉木悦子、松本雅則、石西綾美、松本吉史、冨永麻理恵、工藤理沙、安達聡介、生野寿史、高桑好一、宮腰淑子、小堺貴司、小林弘典、牛木隆志、柴崎康彦、増子正義、瀧澤淳、成田美和子、曽根博仁、西條康夫「妊娠を契機に診断された Upshaw-Schulman 症候群症例における第二子妊娠の周産期管理」第 37 回日本血栓止血学会学術集会、2015 年 5 月 22 日、甲府市、山梨県

36）金谷秀平、小川孔幸、平形絢子、柳澤邦雄、石埼卓馬、三原正大、内藤千晶、半田寛、早川正樹、石西綾美、松本雅則、野島美久、「若年性脳梗塞を契機に診断された高ホモシステイン血症合併 Upshaw-Schulman 症候群の 1 例」第 37 回日本血栓止血学会学術集会、2015 年 5 月 22 日、甲府市、山梨県

37）内藤千晶、小川孔幸、柳澤邦雄、石埼卓馬、三原正大、半田寛、石西綾美、早川正樹、松本雅則、野島美久、「腹部大動脈瘤切迫破裂術後に重症意識障害で発症した血栓性血小板減少性紫斑病の 1 例」第 37 回日本血栓止血学会学術集会、2015 年 5 月 22 日、甲府市、山梨県

38） 吉井由美、藤村吉博、石西綾美、堀勇二、早川正樹、車谷典男、Charles L Bennett、松本雅則「血小板輸血は後天性 TTP の予後を悪化させるか？」第 37 回日本血栓止血学会学術集会、2015 年 5 月 22 日、甲府市、山梨県

39）狩野泰輝、松下文雄、浜子二治、松本雅則、藤村吉博、近藤一直、松井太衛、「ヒト VWF に存在する ABO(H)血液型抗原の付加経路の解析」第 37 回日本血栓止血学会学術集会、2015 年 5 月 22 日、甲府市、山梨県

40） 山下真理子、松本雅則、石西綾美、早川正樹、吉田瑶子、藤村吉博、緒方奈保子、「滲出性加齢黄斑変性患者における Factor H と von Willebrand 因子による病態解析」第 37 回日本血栓止血学会学術集会、2015 年 5 月 22 日、甲府市、山梨県

41） 高岸波穂、堀有沙、浜子二治、松下文雄、松本雅則、早川正樹、藤村吉博、狩野泰輝、近藤一直、松井太衛、「変異導入組換えボトロセチン-2 を用いた血小板凝集の制御」第 37 回日本血栓止血学会学術集会、2015 年 5 月 23 日、甲府市、山梨県

42） 隅志穂里、長谷川真弓、辻内智美、門池真弓、下村志帆、前田美和、早川正樹、松本雅則「血漿分画製剤による副作用の検討」第 63 回

日本輸血・細胞治療学会総会、2015 年 5 月 29 日、東京都

43）Mansouri M, Matsumoto M, Cermakova Z, Friedman K, George J, Hrachovinova I, Knöbl P, Kokame K, von Krogh AS, Schneppenheim R, Vesley S, Fujimura Y, Lämmle B,Johanna A, Hovinga K.“Hereditary TTP-a young patient population with high prevalence of arterial thromboembolic events. First results from the hereditary TTP registry”XXV Congress of the International Society on Thrombosis and Haemostasis and 61st Annual SSC Meeting、2015 年 6 月 23 日、Toronto/Canada

44）Matsui T, Hori A, Hamako J, Matsushita F, Takagishi N, Kondo K, Kano T, Hayakawa M, Matsumoto M, Fujimura Y.“Regulation of VWF-GPIB interaction with modified recombinant botrocetin.”XXV Congress of the International Society on Thrombosis and Haemostasis and 61st Annual SSC Meeting、2015 年 6 月 23 日、Toronto/Canada

45）Yamashita M, Matsumoto M, Isonishi A, Yoshida Y, Hayakawa M, Fujimura Y, Ogata N.“Analysis of plasma von willebrand factor and complement factor H polymorphisms in patients with age-related macular degeneration.”XXV Congress of the International Society on Thrombosis and Haemostasis and 61st Annual SSC Meeting、2015 年 6 月 23 日、Toronto/Canada

46）Nishigori N, Matsumoto M, Koyama F, Hayakawa M, Hatakeyama K, Fujimura Y, Nakajima Y.“Analysis of oxaliplatin-based chemotherapy induced liver injury in patients with advanced colorectal cancer with special references to von willbrand factor.”XXV Congress of the International Society on Thrombosis and Haemostasis and 61st Annual SSC Meeting、2015 年 6 月 23 日、Toronto/Canada

47）Yoshii Y, Yagi H, Hayakawa M, Isonishi A, Yoshida N, Fujimura Y, Matsumoto M.“Characteristics and outcomes in 247 patients with ADAMTS13 activity-deficient primary acquired TTP”第 77 回日本血液学会学術集会、2015 年 10 月 16 日、金沢市、石川県

48）Miyakawa Y, Imada K, Ichinohe T, Yamane Y, Nishio K, Abe T, Fujimura Y, Matsumoto M, Okamoto S.“Investigator-initiated clinical trial of rituximab for thrombotic thrombocytopenic purpura”第 77 回日本血液学会学術集会、2015 年 10 月 16 日、金沢市、石川県

49）松本雅則、教育講演、「TMA の診断と治療」第 77 回日本血液学会学術集会、2015 年 10 月 16 日、金沢市、石川県

50) 松本雅則、シンポジウム「血栓性微小血管症（TMA ）～診断と輸血療法を含めた治療法～」第 22 回日本輸血・細胞治療学会秋季シンポジウム、2015 年 10 月 23 日、長野県

H．知的財産権の出願・登録状況

（予定を含む）

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

図．日本人aHUS患者73人66家系に見られる変異

遺伝子異常部位 アステリスクは論文で既報の変異	患者数 （）内は家族構成員も含めた数			HGVDでのminor allele frequency
C3: I1157T*	14家系	21症例	（28人）	-
THBD: D486Y*	4家系	4症例	（6人）	0.007
CFH: Y1058H,V1060L	4家系	4症例		0.015
CFH: R1215Q*	2家系	2症例	（5人）	-
C3: S562L	2家系	2症例	（4人）	0.008
CFB:R74H	2家系	2症例	（3人）	0.012
MCP: A311V	2家系	2症例		0.008
MCP: T98I	1家系	2症例		0.005
THBD: T500M	1家系	1症例	（3人）	-
MCP: Y189D*	1家系	1症例	（3人）	-
C3: R425C	1家系	1症例	（3人）	0.005
CFH: E1198V	1家系	1症例	（3人）	-
CFH:H651Y	1家系	1症例	（3人）	-
C3: K1105Q	1家系	1症例	（3人）	-
CFB: N331D	1家系	1症例	(2人)	-
MCP: P195S	1家系	1症例	（2人）	-
MCP: A359V	1家系	1症例	（2人）	-
THBD: V231I	1家系	1症例	（2人）	-
THBD: R403K	1家系	1症例		0.005
CFH: R1215G*	1家系	1症例		-
CFH: S1191W*	1家系	1症例	（3人）	-
C3: E1160K	1家系	1症例		-
CFHR1 (homo) / CFHR3 (hetero)欠損*	1家系	1症例		-
MCP: N170Mfs*9 (homo)	1家系	1症例		-
C3:P214S	1家系	1症例		-
DGKE: L24Cfs*145 , IVS9-2 (compound hetero)	1家系	1症例		-

aHUS患者 73人

変異保有者は 44人（60%）

HGVDで登録されている変異を除外

変異保有者は 35人（48%）

Ⅲ．　研究成果の刊行に関する一覧表

別紙４

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Sawai T, Nangaku M, Ashida A, Fujimaru R, Hataya H, Hidaka Y, Kaname S, Okada H, Sato W, Yasuda T, Yoshida Y, Fujimura Y, Hattori M, Kagami S.	Diagnostic criteria for atypical hemolytic uremic syndrome proposed by the Joint Committee of the Japanese Society of Nephrology and the Japan Pediatric Society.	Clin Exp Nephrol	18(1)	4－9	2014
Kanakura Y, Miyakawa Y, Wilde P, Smith J, Achenbach H, Okamoto S.	Phase III, single-arm study investigating the efficacy, safety, and tolerability of anagrelide as a second-line treatment in high-risk Japanese patients with essential thrombocythemia.	Int J Hematol.	100(4)	353-360	2014
Matsukuma E, Imamura A, Iwata Y, Takeuchi T, Yoshida Y, Fujimura Y, Fan X, Miyata T, Kuwahara T	Postoperative atypical hemolytic uremic syndrome associated with complement C3 mutation.	Case Rep Nephrol	784943	5	2014
Matsumoto T, Fan X, Ishikawa E, Ito M, Amano K, Toyoda H, Komada Y, Ohishi K, Katayama N, Yoshida Y, Matsumoto M, Fujimura Y, Ikejiri M, Wada H, Miyata T	Analysis of patients with atypical hemolytic uremic syndrome treated at the Mie University Hospital: concentration of C3 p.I1157T mutation.	Int J Hematol	100(5)	437-442	2014
Morioka M, Matsumoto M, Saito M, Kokame K, Miyata T, Fujimura Y	A first bout of TTP triggered by herpes simplex infection in a 45-year-old nonparous female with Upshaw-Schulman syndrome.	Blood Transfus	12 suppl 1	s153-155	2014

Eura Y, Kokame K, Takafuta T, Tanaka R, Kobayashi H, Ishida F, Hisanaga S, Matsumoto M, Fujimura Y, Miyata T	Candidate gene analysis using genomic quantitative PCR: identification of ADAMTS13 large deletions in two patients with Upshaw-Schulman syndrome.	Mol Genet Genomic Med	2(3)	240-244	2014
Sorvillo N, Kaijen P H, Matsumoto M, Fujimura Y, van der Zwaan C, Verbij FC, Pos W, Fijnheer R, Voorberg J, Meijer AB	Identification of N-linked glycosylation and putative O-fucosylation, C-mannosylation sites in plasma derived ADAMTS13.	J Thromb Haemost	12	670-679	2014
Ohta T, Urayama K, Tada Y, Furue T, Imai S, Matsubara K, Ono H, Sakano T, Jinno K, Yoshida Y, Miyata T, Fujimura Y	Eculizumab in the treatment of atypical hemolytic uremic syndrome in an infant leads to cessation of peritoneal dialysis and improvement of severe hypertension.	Pediatr Nephrol	30(4)	603-608	2015
Hisano M, Ashida A, Nakano E, Suehiro M, Yoshida Y, Matsumoto M, Miyata T, Fujimura Y, Hattori M	Autoimmune-type atypical hemolytic uremic syndrome treated with eculizumab as first-line therapy.	Pediatr Int	57(2)	313-317	2015
Yoshida Y, Miyata T, Matsumoto M, Shirotani-Ikejima H, Uchida Y, Oyama Y, Kokubo T, Fujimura Y	A Novel quantitative hemolytic assay coupled with restriction fragment length polymorphisms analysis enabled early diagnosis of atypical hemolytic uremic syndrome and identified unique predisposing mutations in Japan.	PLoS ONE	10(5)	e0124655	2015
Imamura H, Konomoto T, Tanaka E, Hisano S, Yoshida Y, Fujimura Y, Miyata T, Nunoi H	Familial C3 glomerulonephritis associated with mutations in the gene for complementfactor B.	Nephrol Dial Transplant	30(5)	862-864	2015
Miyata T	GWA study for ADAMTS13 activity	Blood	125(25)	3833-3834	2015

Miyata T, Uchida Y, Ohta T, Urayama K, Yoshida Y, Fujimura Y	Atypical haemolyticuremic syndrome in a Japanese patient with *DGKE* genetic mutations.	Thromb Haemost	114(4)	862-863	2015
Ogawa Y, Matsumoto M, Sadakata H, Isonishi A, Kato S, Nojima Y, Fujimura Y	A unique case involving a female patient with Upshaw-Schulman syndrome: low titers of antibodies against ADAMTS13 prior to pregnancy disappeared after successful deliver.	Transfus Med and Hemotherapy	42(1)	59-63	2015
Yada N, Fujioka M, Bennett C, Hayakawa M, Matsumoto M, Inoki K, Miki T, Watanabe A, Yoshida T, Fujimura Y	The STEC-HUS followed by acute encephalopathy in ayoung girl was favorably treated on a basis of hemodiafiltration, steroid pulse, and soluble thrombomodulin, under plasma exchange.	Clin Case Reports	3(4)	208-212	2015
Kato S, Tanaka M, Isonishi A, Matsumoto M, Samori T, Fujimura Y	A rapid, fully automated and highly sensitive ADAMTS13 gold particle immunoassay using aroutine biochemistry analyser.	Br J Haematol	171(4)	655-658	2015
Isonishi A, Bennett CL, Plaimauer B, Scheiflinger F, Matsumoto M, Fujimura Y	Poor-responder to plasma exchange therapy in acquired TTP is associated with ADAMTS13 inhibitor boosting: Visualization of an ADAMTS13-inhibitor complex, and its proteolytic clearance from plasma.	Transfusion	55(10)	2321-2330	2015

Sei Y, Mizuno M, Suzuki Y, Imai M, Higashide K, Harris CL, Sakata F, Iguchi D, Fujiwara M, Kodera Y, Maruyama S, Matsuo S, Ito Y.	Expression of membrane complement regulators, CD46, CD55 and CD59, in mesothelial cells of patients on peritoneal dialysis therapy.	Mol Immunol	65(2)	302-309	2015
Ito N, Hataya H, Saida K, Amano Y, Hidaka Y, Motoyoshi Y, Ohta T, Yoshida Y, Terano C, Iwasa T, Kubota W, Takada H, Hara T, Fujimura Y, Ito S	Efficacy and safety of eculizumab in childhood atypical hemolytic uremic syndrome in Japan.	Clin Exp Nephrol	in press		
Nishigori N, Matsumoto M, Koyama F, Hayakawa M, Hatakeyayama K, Ko S, Fujimura Y, Nakajima Y	von Willebrand Factor-rich platelet thrombi in the liver cause sinusoidal obstruction syndrome following oxaliplatin-based chemotherapy.	PLoS ONE	10(11)	e0143136	2015
Miyakawa Y, Katsutani S, Yano T, Nomura S, Nishiwaki K, Tomiyama Y, Higashihara M, Shirasugi Y, Nishikawa M, Ozaki K, Abe T, Kikuchi K, Kanakura Y, Fujimura K, Ikeda Y, Okamoto S.	Efficacy and safety of rituximab in Japanese patients with relapsed chronic immune thrombocytopenia refractory to conventional therapy.	Int J Hematol	102(6)	654-661	2015
Fan X, Kremer Hovinga JA, Shirotani-Ikejima H, Eura Y, Hirai H, Honda S, Kokame K, Taleghani MM, von Krogh AS, Yoshida Y, Fujimura Y, Lämmle B, Miyata T.	Genetic variations in complement factors in patients with congenital thrombotic thrombocytopenic purpura with renal insufficiency.	Int J Hematol	in press		
宮田敏行、中村敏子	徹底ガイド DICのすべて2014-2015 IV章病態生理と病理、「補体反応」	救急・集中治療	第26巻、第5・6号	668-673	2014

加藤秀樹、吉田瑶子、南学正臣	補体・凝固関連aHUSの病態	日本腎臓学会誌	第56巻、第7号	1058-1066	2014
宮田敏行、瀬谷 司	特集 補体のすべて、機能から病態まで「疾患から見た補体の活性化と制御」	血液フロンティア	第25巻、第9号	23-32	2015
南学 正臣、吉田 瑤子、加藤 秀樹	TMA HUSとatypical HUS	日本内科学会雑誌	第104巻、第9号	1959-1963	2015

※その他、平成28年2月に日本腎臓学会と日本小児科学会より正式に公表された「非典型溶血性尿毒症症候群診療ガイド」を添付

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SPECIAL ARTICLE

Diagnostic criteria for atypical hemolytic uremic syndrome proposed by the joint committee of the Japanese society of nephrology and the Japan pediatric society

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Abstract Atypical hemolytic uremic syndrome (aHUS) is rare and comprises the triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute kidney injury. Recently, abnormalities in the mechanisms underlying complement regulation have been focused upon as causes of aHUS. The prognosis for patients who present with aHUS is very poor, with the first aHUS attack being associated with a mortality rate of ~25 %, and with ~50 % of cases resulting in end-stage renal disease requiring dialysis. If treatment is delayed, there is a high risk of this syndrome progressing to renal failure. Therefore, we have developed diagnostic criteria for aHUS to enable its early diagnosis and to facilitate the timely initiation of appropriate treatment. We hope these diagnostic criteria will be disseminated to as many clinicians as possible and that they will be used widely.

Keywords Atypical hemolytic uremic syndrome · Thrombotic microangiopathy · Complement dysregulation · Alternative complement pathway · ADAMTS13

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Introduction

Hemolytic uremic syndrome (HUS) is characterized by the triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute kidney injury (AKI) [1]. Approximately 90 % of pediatric patients develop this syndrome after infection with *Shigella dysenteriae*, which produces true Shiga toxins, or *Escherichia coli*, some strains of which produce Shiga-like toxins. Shiga toxin was originally called verotoxin because Vero cells derived from the kidney epithelial cells of the African green monkey are hypersensitive to this toxin [2]. Subsequently, other toxins were called Shiga-like toxin because of their similarities to Shiga toxin in terms of their antigenicity and structure. Shiga-like toxin-1 differs from Shiga toxin by only 1 amino acid, whereas Shiga-like toxin-2 shares 56 % sequence homology with Shiga-like toxin-1. Although Shiga-like toxin-producing *E. coli*-HUS (STEC-HUS) strains most often trigger HUS, certain Shiga toxin-secreting strains of *S. dysenteriae* can also cause HUS. They are currently known as the Shiga toxin family, and the terms are often used interchangeably. HUS occurring from infection with STEC-HUS was formerly called diarrhea + HUS (D + HUS) or typical HUS.

In contrast, HUS that is not related to Shiga toxins and accounts for ~10 % of all HUS cases, is called atypical HUS (aHUS). Although STEC-HUS is relatively common in children, aHUS occurs in individuals of all ages and is often familial. The prognosis is very poor, with the first aHUS attack being associated with a mortality rate of ~25 %, and with ~50 % of cases resulting in end-stage renal disease requiring dialysis [3].

In recent years, abnormalities in the mechanisms underlying complement regulation have been focused on as causes of aHUS. Various genetic abnormalities in complement regulatory factors, including complement factor H, have been noted in 50–60 % of patients. The analysis of the pathology underlying this condition is currently progressing rapidly [4].

The differential diagnosis of aHUS from STEC-HUS or thrombotic thrombocytopenic purpura (TTP), another form of thrombotic microangiopathy (TMA) caused by a deficiency of ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), is not necessarily easy at the early stages of disease onset. However, if treatment is delayed, there is a high risk of this syndrome progressing to renal failure. Therefore, the Joint Committee of the Japanese Society of Nephrology and the Japan Pediatric Society (JSN/JPS) has developed diagnostic criteria for aHUS to enable its early diagnosis and to facilitate the timely initiation of appropriate treatment [5, 6]. We hope that the diagnostic criteria presented in this report will become familiar to as many clinicians as possible and that they will be used widely.

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Definition of aHUS

aHUS is a type of TMA that differs from STEC-HUS and TTP, with the latter being caused by markedly reduced ADAMTS13 activity. aHUS is a syndrome characterized by the triad of microangiopathic hemolytic anemia, thrombocytopenia, and AKI, which is similar to STEC-HUS.

Guidelines for the diagnosis of aHUS

Definitive diagnosis

A definitive diagnosis of aHUS is made when the triad of microangiopathic hemolytic anemia, thrombocytopenia, and AKI is present. The disease should not be associated with Shiga toxins, and TTP should also be excluded.

The Joint Committee of the JSN/JPS defined microangiopathic hemolytic anemia based on a hemoglobin (Hb) level of <10 g/dL. The presence of microangiopathic hemolytic anemia should be confirmed based on increased serum lactate dehydrogenase levels, a marked decrease in serum haptoglobin levels, and the presence of red blood cell fragments in a peripheral blood smear.

Thrombocytopenia is defined as a platelet (PLT) count of <150,000/μL.

The definition of AKI has been updated, with the most recent definition given by the international guidelines group, the Kidney Disease: Improving Global Outcomes that integrates both the Risk, Injury, Failure, Loss, End-stage kidney disease and the Acute Kidney Injury Network classifications to facilitate identification. Thus, we recommend diagnosis based on the most recent guidelines, along with the following definitions. For pediatric cases, the serum creatinine should be increased to a level that is 1.5fold higher than the serum creatinine reference values based on age and gender issued by the Japanese Society for Pediatric Nephrology [7]. For adult cases, the diagnostic criteria for AKI should be used.

Guidelines for the diagnosis of aHUS

Definitive diagnosis

A definitive diagnosis of aHUS is made when the triad of microangiopathic hemolytic anemia, thrombocytopenia,

Table 1 Definitions of microangiopathic hemolytic anemia, thrombocytopenia, and AKI that have been established by the joint committee of the JSN/JPS

Microangiopathic hemolytic anemia	Thrombocytopenia	Acute kidney injury
Defined as an Hb level <10 g/dL Presence confirmed based on: Increased serum LDH levels Marked decreases in serum haptoglobin levels The presence of red blood cell fragments in a peripheral blood smear	Defined as a PLT count <150,000/μL	The most recent AKI definition is provided by the international guideline group, the KDIGO, integrating the RIFLE and AKIN classifications to facilitate identification. Thus, diagnosis should be based on the most recent guidelines, and the following definitions should be used. Pediatric cases: Serum creatinine should be increased to a level that is 1.5fold higher than the serum creatinine reference values based on age and gender issued by the Japanese Society for Pediatric Nephrology [7]. Adult cases: Diagnostic criteria for AKI should be used

Hb hemoglobin, *LDH* lactate dehydrogenase, *PLT* platelet, *AKI* acute kidney injury, *KDIGO* kidney disease: improving global outcomes, *RIFLE* risk, injury, failure, loss, end-stage kidney disease, *AKIN* acute kidney injury network

and AKI is present. The disease should have no association with Shiga toxins, and TTP should also be excluded. Table 1 presents the definitions of microangiopathic hemolytic anemia, thrombocytopenia, and AKI that are established by the Joint Committee of the JSN/JPS.

Probable diagnosis

A probable diagnosis of aHUS is made when 2 of the following 3 conditions are found: microangiopathic hemolytic anemia, thrombocytopenia, and AKI. The disease should have no association with Shiga toxins and TTP should be excluded.

Applicability of these diagnostic criteria

When we applied these diagnostic criteria to the Nara Medical University (NMU) TMA cohort, 15 out of 37 individuals who had all the data required for the assessment were diagnosed as having definitive aHUS. Since the data were recorded at one time point only, we speculate that the sensitivity of the diagnostic criteria would increase if we could assess data from multiple time points. The cut-off value for anemia, defined as an Hb level of <10 g/dL, and the cut-off value for thrombocytopenia, defined as a PLT count of <150,000/μL, are equivalent to those employed by the International Registry of Recurrent and Familial HUS/TTP [8]. We had considered using a cut-off value of a PLT count <100,000/μL for thrombocytopenia to reflect that used in the diagnostic criteria for STEC-HUS by the Japanese Society for Pediatric Nephrology (2000), but we only found 1 patient with a PLT count between 100,000 and 150,000/μL in the NMU cohort. Therefore, it is likely that this difference will not have a large impact on the sensitivity or specificity of our diagnostic criteria. Our diagnostic criteria include the category of "Probable" aHUS because we believe that this tentative diagnosis will help in the early diagnosis of aHUS and avoid delays in developing appropriate therapeutic approaches for patients with aHUS.

Evaluation of inappropriate complement activation

Abnormalities in complement regulation are among the main causes of aHUS. The diagnosis of aHUS that is caused by inappropriate complement activation has become more critical because eculizumab, a humanized anti-C5 monoclonal antibody, has been shown to be an effective therapeutic modality [9] that has been approved for the treatment of aHUS patients in Europe and the United States. Recently, Fan and colleagues evaluated genotype–phenotype relationships in 10 Japanese patients with aHUS and identified potentially causative mutations in complement factor H, C3, membrane cofactor protein, and thrombomodulin in 8 of the patients [10]. However, the definitive diagnosis of inappropriate complement activation in aHUS patients is difficult because some patients show normal serum levels of complement components [11] and there are a number of complement regulatory proteins, making it difficult to decide which complement regulatory protein is responsible for a particular patient developing aHUS.

Excluding Shiga toxin-producing *E. coli* infection

STEC-HUS is characterized by diarrhea accompanied by bloody stools. However, diarrhea may also be present in some aHUS cases. Diarrhea in aHUS can be a manifestation of ischemic colitis. In addition, enteritis that is not caused by STEC can trigger aHUS. Therefore, a diagnosis of STEC-HUS cannot be made based on symptoms alone, and the earlier nomenclature that used "D + HUS" to correspond with STEC-HUS and "D-HUS" to correspond

with aHUS is not used at present [11]. The involvement of Shiga toxins should be confirmed by stool culture, the direct detection of Shiga toxins, or the detection of anti-lipopolysaccharide-IgM antibodies.

Excluding TTP

Conventionally, TTP has been diagnosed based on the classic pentad (microangiopathic hemolytic anemia, thrombocytopenia, labile psychoneurotic disorder, fever, and renal failure). However, the discovery of ADAMTS13 led to the finding that 60–90 % of patients with TTP have a marked reduction in the activity of ADAMTS13, to a level of <5 %, regardless of race. Therefore, when diagnosing aHUS, patients who have markedly reduced levels of ADAMTS13 activity (<5 %) should be diagnosed as having TTP, thereby ruling out a diagnosis of aHUS. However, some patients may show the classic TTP pentad and have normal or slightly reduced levels of ADAMTS activity. Therefore, if a patient has levels of ADAMTS13 activity ≥5 %, a differential diagnosis of aHUS or TTP may be necessary to account for other clinical symptoms.

Excluding TMA caused by other distinct factors

Diseases that evidently cause a clinical state of TMA, including disseminated intravascular coagulation, sclerodermatous kidney, and malignant hypertension, should be excluded when diagnosing aHUS.

When a probable case of aHUS is suspected

When a probable case of aHUS is suspected, samples that are necessary to determine the appropriate diagnosis should be collected, and the therapeutic strategy should be established after consultation with an institution that has extensive experience of managing aHUS cases.

Cases where aHUS should be strongly suspected

If there are features that are characteristic of HUS, aHUS should be strongly suspected if the following criteria are fulfilled, regardless of the presence of diarrhea: the patient is younger than 6 months of age; time of onset is unclear (latent onset); the patient has a history of HUS (recurrent case); the patient has a history of anemia of unknown cause; recurrent HUS after kidney transplantation; the patient has a family history of HUS (excluding cases of food poisoning); and, the patient has no diarrhea or bloody stools.

Classification of aHUS causes, excluding TTP caused by the ADAMTS13 defect

Table 2 classifies the causes of aHUS and presents methods to determine the causes.

Discussion

Nineteen years after Gasser et al. [1] reported HUS, an interesting report was published in the *Lancet* [10]. This report indicated that although C3-predominant activity is initiated in the blood vessels in TMA patients, this is not observed in typical cases of HUS, suggesting that complement activation is involved in aHUS onset [12]. Subsequently, numerous researchers have elucidated further information on the pathology of aHUS. At present, the reported causes of aHUS include, complement regulation abnormalities, cobalamin metabolism disorder, infection with *Streptococcus pneumoniae* and other microorganisms, drugs, pregnancy, and autoimmune diseases.

The complement system plays an important role as part of the immune systems of living organisms. It is activated via 3 pathways, the classical, alternative, and lectin pathways. As a result of the activation of the host's alternative and classical pathways, C5b-9, a membrane attack complex, is generated and destroys cells by forming transmembrane pores. The alternative pathway is involved in the onset of aHUS. Unlike the classical and lectin pathways, activation of the alternative pathway does not require initiators; it is continuously activated by the spontaneous hydrolysis of C3.

When complement proteins are inappropriately activated, there is a risk of inducing cell dysfunction within the host itself. Thus, humoral factors in the circulating plasma and several plasma membrane-bound factors are involved in the regulation of complement activation and act at various stages, such as the inactivation of C3b or C4b, and the inhibition of the generation of membrane attack complexes. The regulators involved in the alternative pathway include complement factors H and I, which are humoral factors, and membrane cofactor protein and thrombomodulin, which are membrane-bound factors. If these factors are abnormal, the subsequent failure of regulation will hyperactivate the complement proteins, leading to the onset of aHUS. Some cases of aHUS develop after trigger events, for example, infections of the respiratory tract and the gastrointestinal tract, and it is likely that activation of the complement cascade by these trigger events and the

Table 2 Classification and determination of the causes of aHUS, excluding TTP caused by the ADAMTS13 defect

Cause of aHUS	Method to determine the cause
Complement regulation abnormality	Hemolysis test, quantification of complement proteins and complement regulatory proteins, and gene analysis. Even if the amounts of complement proteins and complement regulatory proteins are within the normal ranges, it does not serve as a basis for excluding complement-related aHUS
(i) Congenital	
Genetic mutations of complement proteins, factor H, factor I, membrane cofactor protein, C3, factor B, and thrombomodulin	
(ii) Acquired	
Production of autoantibodies, including anti-factor H antibody	Detection of anti-factor H antibody by ELISA, western blot, etc.
(2) Cobalamin metabolism disorder	Age at onset should be considered (<6 months old), and hypomethioninemia or hyperhomocysteinemia is detected on plasma amino acid analysis
(3) Infection	Definitive diagnosis by identification of pathogenic microorganisms and serological examination
(i) Pneumococcus	
(ii) Human immunodeficiency virus	
(iii) Pertussis	
(iv) Influenza	
(v) Varicella	
(4) Drug-induced	Identification of the drug
(i) Anticancer drugs	
(ii) Immunomodulatory drugs	
(iii) Antiplatelet drugs	
(5) Pregnancy-related	
(i) Hemolysis, elevated liver enzymes, low platelet counts (HELLP) syndrome	
(ii) Eclampsia	
(6) Autoimmune disease, collagen disease	Definitive diagnosis by autoantibody test, antiphospholipid antibody test, and serological examination
(i) Systemic lupus erythematosus	
(7) Bone-marrow transplant, organ transplant-related	
(8) Others	

aHUS atypical hemolytic uremic syndrome, *ELISA* enzyme-linked immunosorbent assay

subsequent amplification of complement activation by the alternative pathway cannot be regulated in patients with deficiencies in complement regulation. Gain-of-function mutations in C3 and complement factor B, which are complement-activating factors, also cause hyperactivation of complement proteins and, ultimately, aHUS.

It has been reported that ~50 % of aHUS patients have genetic abnormalities in complement regulatory factors, including complement factor H. The frequency of the presence of certain mutations among aHUS cases, responsiveness to plasma therapy, prognosis of kidney function, and the recurrence rate after kidney transplantation, vary depending on the type of genetic abnormalities present [13]. Although plasmapheresis within 24 h of confirmation of the diagnosis has been recommended as the initial treatment for aHUS [14], its effects are not always satisfactory. The mortality or incidence of end-stage renal disease is considered to be between 70 and 80 %, and the recurrence rate after kidney transplantation may be as high as 80–90 %, particularly in patients with abnormal complement factor H, which is the most frequent abnormality [15].

In 2011, eculizumab (Soliris®, Alexion Pharmaceuticals), a terminal complement inhibitor, was approved as a new drug for the treatment of aHUS in Europe and the US. Eculizumab is a humanized recombinant immunoglobulin G2/4 monoclonal antibody directed against the complement component C5, which was developed as a treatment for paroxysmal nocturnal hemoglobinuria. By binding to complement component C5, the drug inhibits the generation of C5a and C5b-9, and thus subsequently inhibits the complement system.

There are a number of reports stating that only HUS that is associated with complement regulation abnormalities is defined as aHUS. On the basis of the current diagnostic criteria, we have defined aHUS to include all types of HUS that are not related to Shiga toxins or other distinct causes. In cases where aHUS is associated with complement

dysregulation, the introduction of eculizumab may markedly change therapeutic strategies. It should be noted, however, that recommendations of specific therapeutic modalities are beyond the scope of the current diagnostic criteria. However, in cases where complement dysregulation is confirmed as the cause, treatment with eculizumab is established. Thus, it may be desirable to assign HUS associated with complement dysregulation a separate disease name rather than it being classified as "aHUS", as in the case of definitive "complement-mediated TMA".

As described in previous reports, aHUS is a disease that may frequently cause renal failure and be fatal if it is not appropriately diagnosed and treated at the early stages of disease onset. In Japan, aHUS may be misdiagnosed as HUS caused by Shiga toxins because clinicians are not sufficiently aware of aHUS, and consequently, treatment may be delayed. Thus, our diagnostic criteria include the category of "Probable" aHUS to ensure that the clinicians consider aHUS during diagnosis. Many issues should be addressed in the future, including the development of diagnostic strategies to diagnose cases of suspected aHUS, the establishment of insurance coverage for ADAMTS13 activity measurement testing that is necessary to differentiate aHUS from TTP, and the development of treatment guidelines. We hope that our diagnostic criteria will be used widely and will contribute to the diagnosis and treatment of aHUS patients.

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References

1. Gasser C, Gautier E, Steck A, Siebenmann R, Oechslin R. Hemolytic-uremic syndrome: bilateral necrosis of the renal cortex in acute acquired hemolytic anemia. Schweizerische medizinische Wochenschrift. 1955;85(38–39):905–9.
2. Konowalchuk J, Speirs JI, Stavric S. Vero response to a cytotoxin of *Escherichia coli*. Infect Immun. 1977;18:775–9.
3. Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. N Engl J Med. 2009;361(17):1676–87. doi:10.1056/NEJMra0902814.
4. Kavanagh D, Goodship T. Genetics and complement in atypical HUS. Pediatric Nephrol (Berlin, Germany). 2010;25(12):2431–42. doi:10.1007/s00467-010-1555-5.
5. Kagami S, Okada H, Kaname S, Sato W, Nangaku M, Yasuda T, et al. Diagnostic criteria of atypical hemolytic uremic syndrome. Jpn J Nephrol. 2013;55(2):91–3 (in Japanese).
6. Kagami S, Okada H, Kaname S, Sato W, Nangaku M, Yasuda T et al. Diagnostic criteria of atypical hemolytic uremic syndrome. J Jpn Pediatr Soc. 2013; http://www.jpeds.or.jp/uploads/files/saisin_130201.pdf (in Japanese).
7. Uemura O, Honda M, Matsuyama T, Ishikura K, Hataya H, Yata N, et al. Age, gender, and body length effects on reference serum creatinine levels determined by an enzymatic method in Japanese children: a multicenter study. Clin Exp Nephrol. 2011;15(5):694–9. doi:10.1007/s10157-011-0452-y.
8. Noris M, Caprioli J, Bresin E, Mossali C, Pianetti G, Gamba S, et al. Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. Clin J Amer Soc Nephrol. 2010;5(10):1844–59. doi:10.2215/CJN.02210310.
9. Legendre C, Licht C, Muus P, Greenbaum L, Babu S, Bedrosian C, et al. Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. N Engl J Med. 2013;368(23):2169–81. doi:10.1056/NEJMoa1208981.
10. Fan X, Yoshida Y, Honda S, Matsumoto M, Sawada Y, Hattori M, et al. Analysis of genetic and predisposing factors in Japanese patients with atypical hemolytic uremic syndrome. Mol Immunol. 2013;54(2):238–46. doi:10.1016/j.molimm.2012.12.006.
11. Loirat C, Frémeaux-Bacchi V. Atypical hemolytic uremic syndrome. Orphanet J Rare Dis. 2011;6:60. doi:10.1186/1750-1172-6-60.
12. Stühlinger W, Kourilsky O, Kanfer A, Sraer J. Letter: haemolytic-uraemic syndrome: evidence for intravascular C3 activation. Lancet. 1974;2(7883):788–9.
13. Nester C, Thomas C. Atypical hemolytic uremic syndrome: what is it, how is it diagnosed, and how is it treated? Hematology. 2012;2012:617–25. doi:10.1182/asheducation-2012.1.617.
14. Ariceta G, Besbas N, Johnson S, Karpman D, Landau D, Licht C, et al. Guideline for the investigation and initial therapy of diarrhea-negative hemolytic uremic syndrome. Pediatric Nephrol (Berlin, Germany). 2009;24(4):687–96. doi:10.1007/s00467-008-0964-1.
15. Waters A, Licht C. aHUS caused by complement dysregulation: new therapies on the horizon. Pediatric Nephrol (Berlin, Germany). 2011;26(1):41–57. doi:10.1007/s00467-010-1556-4.

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ORIGINAL ARTICLE

Phase III, single-arm study investigating the efficacy, safety, and tolerability of anagrelide as a second-line treatment in high-risk Japanese patients with essential thrombocythemia

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Abstract Essential thrombocythemia (ET) is usually managed by anti-platelet therapy. European guidelines recommend that patients with ET at high risk of developing thrombohemorrhagic events should be placed on cytoreductive therapy (CRT). In Japan, hydroxycarbamide (HC) is the most widely used CRT; however, treatment options for patients who become intolerant or refractory to initial treatment are limited. This study sought to determine the efficacy, safety, and tolerability of anagrelide in high-risk Japanese adults with ET who were intolerant or refractory to their first-line CRT. Fifty-three patients were enrolled in the study. Of those, 67.9 % had a platelet response ($<60 \times 10^4/\mu L$) and 45.3 % achieved normalization of platelet counts ($\leq 40 \times 10^4/\mu L$) on anagrelide therapy. The median time to platelet count response was 98.5 days and the median time to platelet count normalization was 274.0 days. The median daily dose administered was 1.9 mg/day. The most common adverse events observed during anagrelide treatment were anemia, headache, palpitations, and diarrhea. The majority of these were either mild or moderate in severity. Overall, the safety profile of anagrelide in high-risk Japanese patients with ET was consistent with the European Summary of Product Characteristics.

Keywords Essential thrombocythemia · Japan · Anagrelide · Second-line treatment

Introduction

Essential thrombocythemia (ET) is predominantly characterized by thrombocytosis and abnormal megakaryocyte proliferation [1], and its clinical course is often complicated with thromboembolic events. According to European guidelines, it is strongly recommended that cytoreductive therapy (CRT) is initiated in patients who are at high risk of developing thrombotic or hemorrhagic events. High-risk patients can be categorized based upon three criteria: >60 years of age, history of thrombosis, or a high platelet count. Currently, at least one platelet count reading of $>150 \times 10^4/\mu L$ is required by the European LeukemiaNet guidelines to be categorized as a high-risk patient [2]; however, at the time of this study a platelet count of $>100 \times 10^4/\mu L$ was regarded as the cut-off for being a high-risk patient [3].

Hydroxycarbamide (HC) is the most widely used CRT for ET in Japan [4]. It has been suggested that long-term HC use is associated with a higher incidence of secondary leukemia [5]. Thus non-leukemogenic drugs such as anagrelide or interferon-α are treatments of choice for younger patients [2]. However, no compounds are currently licensed

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as a second-line treatment for patients who become intolerant or refractory to their initial CRT in Japan. In Europe, anagrelide is approved as a second-line therapy for patients with ET who are intolerant or refractory to their prior CRT [6]. It is also licensed in the US as a first-line therapy for thrombocythemia in patients with myeloproliferative neoplasms (MPN) [7]. It is important to identify patients who become intolerant or refractory to HC treatment in order to avoid continuation of the drug that may produce intolerable side effects and to stop the use of an ineffective drug in a high-risk disease, but also to manage side effects appropriately to avoid the premature discontinuation of the drug that could otherwise prove efficacious [8].

The aims of this study were to evaluate the efficacy, safety, and tolerability of anagrelide as a second-line therapy in high-risk Japanese patients with ET who were intolerant or refractory to their first-line CRT.

Materials and methods

Study design

Study SPD422-308 (clinicaltrials.gov ID: NCT01214915) was a Phase III, open-label, single-arm study investigating the efficacy, safety, and tolerability of anagrelide in high-risk Japanese adults with ET who were intolerant or refractory to their first-line CRT. The study consisted of three periods: a 28-day screening period, a 12-month treatment period, and a 7-day follow-up period.

Patients

Patients aged 20 years or older were eligible to enroll in the study if they were previously diagnosed with ET according to the World Health Organization (WHO) criteria [1] and were considered at 'high risk' of thrombohemorrhagic events defined according to the anagrelide European Summary of Product Characteristics (SPC) (a platelet count of $>100 \times 10^4/\mu L$, >60 years of age, or history of previous thrombohemorrhagic events), and were refractory or intolerant to their first-line CRT. Refractory patients were defined as having a platelet count $>60 \times 10^4/\mu L$ after 3 months of at least 2 g/day of HC, and intolerant patients were defined as either having a platelet count $>40 \times 10^4/\mu L$ and white blood cell count $<2500/\mu L$ at any dose of HC, or platelet count $>40 \times 10^4/\mu L$ and hemoglobin <10 g/dL at any dose of HC, or the presence of leg ulcers or other mucocutaneous manifestations at any dose of HC, or HC-related fever [8]. All patients provided written, signed, and dated informed consent in order to participate in the study. Patients who were treated within 3 months prior to study entry with anagrelide, or were being treated with anticoagulant therapies, were excluded from the study. In addition, patients with the following were excluded: drug hypersensitivity or intolerance to anagrelide, receiving medication that had phosphodiesterase (PDE) III inhibitory properties, receiving medication that could affect their ET or the action of anagrelide, abnormal laboratory values, cardiac disease, hepatitis B, hepatitis C, human immunodeficiency virus, renal impairment, clinically significant malignancies or neoplasia, current or recurrent disease that might influence the action of anagrelide, current or relevant physical or psychiatric illness that might be affected by anagrelide treatment, a history of alcohol or other substance abuse in the 2 years prior to enrollment, or had been enrolled in a clinical study within the last 30 days. The study was conducted in accordance with the International Conference on Harmonization guidelines for Good Clinical Practice.

Treatment

The starting dose of anagrelide was 0.5 mg/day twice daily (total dose 1.0 mg/day) as recommended in the anagrelide European SPC and as previously determined in a Phase I/II study in Japanese patients with ET to be clinically effective at reducing platelet counts [9]. This dose had to be maintained for at least 1 week prior to titration. Anagrelide titration was designed to achieve a response at the lowest effective dose and was assessed on an individual basis. Dosage increments could not exceed 0.5 mg/day in any one week and total daily doses could not exceed 10 mg. Patients could receive three to four doses of anagrelide per day following the first week of dosing if they were required to receive more than 5 mg/day, i.e. single doses could not exceed 2.5 mg/day. Following a protocol amendment, patients were allowed to continue HC treatment for the first month of the study after baseline measurements, if the treating physician determined that it was required to control platelet counts, while anagrelide was titrated to an effective dose.

Study objectives

The primary objective of the study was to evaluate the proportion of patients who had a response in platelet count ($<60 \times 10^4/\mu L$). The secondary objectives were to evaluate the proportion of patients who: (a) achieved a 50 % reduction in platelet count vs. their baseline values and (b) reached normalization in platelet count ($\leq 40 \times 10^4/\mu L$). To meet the definition of response or normalization, platelet counts had to be consistent across consecutive visits for at least 4 weeks following at least 3 months of anagrelide treatment (the same criteria were used to identify those patients who achieved a 50 % reduction in their platelet counts). In addition, patients were categorized according to their baseline platelet count (i.e. $\geq 60 \times 10^4/$