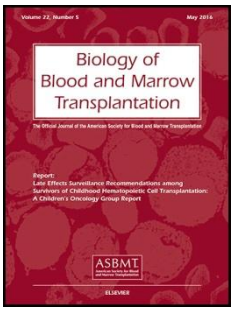
Accepted Manuscript



Title: Acquired Complement Regulatory Gene Mutations and Hematopoietic Stem Cell Transplant-Related Thrombotic Microangiopathy

Author: Gianluigi Ardissino, Stefania Salardi, Silvia Berra, Giacomo Colussi, Massimo Cugno, Marco Zecca, Fabio Giglio, Jacopo Peccatori, Elisa Diral, Francesca Tel, Alberto Clivio, Silvana Tedeschi

PII: S1083-8791(17)30460-3
DOI: http://dx.doi.org/doi: 10.1016/j.bbmt.2017.05.013
Reference: YBBMT 54674

To appear in: *Biology of Blood and Marrow Transplantation*

Received date: 10-3-2017
Accepted date: 9-5-2017

Please cite this article as: Gianluigi Ardissino, Stefania Salardi, Silvia Berra, Giacomo Colussi, Massimo Cugno, Marco Zecca, Fabio Giglio, Jacopo Peccatori, Elisa Diral, Francesca Tel, Alberto Clivio, Silvana Tedeschi, Acquired Complement Regulatory Gene Mutations and Hematopoietic Stem Cell Transplant-Related Thrombotic Microangiopathy, *Biology of Blood and Marrow Transplantation* (2017), http://dx.doi.org/doi: 10.1016/j.bbmt.2017.05.013.

TITLE PAGE

Title: Acquired Complement Regulatory Gene Mutations and Hematopoietic Stem Cell Transplant-Related Thrombotic Microangiopathy

Running Title: Acquired complement gene mutation with HSCT

Authors: Gianluigi Ardissino[1], MD, PhD, Stefania Salardi[2], MS, Silvia Berra[3], MS, PhD, Giacomo Colussi[4], MD, PhD, Massimo Cugno[5], MD, PhD, Marco Zecca[6], MD, PhD, Fabio Giglio[7], MD, Jacopo Peccatori[7], MD, Elisa Diral[8], MD, Francesca Tel[1], MD, Alberto Clivio[3], MS, PhD, and Silvana Tedeschi[2], MS.

Affiliations:

[1]Center for HUS Control, Prevention and Management and [2]Laboratory of Medical Genetics, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milano, Italy; [3]Dipartimento di Scienze Biomediche e Cliniche "Luigi Sacco", Università di Milano, Italy; [4]S.C. Nefrologia, Grande Ospedale Metropolitano Niguarda, Milano, Italy; [5]Medicina Interna, Dipartimento di Fisiopatologia Medico Chirurgica, Università degli Studi di Milano, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milano, Italy; [6]Pediatric Hematology/Oncology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; [7]Hematology and Bone Marrow Transplantation Unit, IRCCS San Raffaele Scientific Institute, Milano, Italy, [8]Divisione di Ematologia e CTA, Ospedale San Gerardo, Monza, Italy.

Corresponding author

Dr. Gianluigi Ardissino

Center for HUS Control, Prevention and Management, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico

Via della Commenda 9, 20122 Milano, Italy

phone number: +39.3356065755

e-mail: ardissino@centroseu.org

HIGHLIGHTS

- HSCT-TMA maybe related to complement disregulation
- Genetic complement disregulation in HSCT may be acquired from the donor
- The screening of donors for genetic complement disregulation may be useful in HSCT

Abstract

Hematopoietic stem cell transplant-related thrombotic microangiopathy (HSCT-TMA) is a severe complication whose pathophysiology is unknown.

We describe 6 patients in which the disease was associated with complement regulatory gene abnormalities received from their respective donors. It is suggested that mutated and transplanted monocyte-derived cells are responsible for production of abnormal proteins, complement dysregulation and, ultimately, for the disease. This observation might have important drawbacks as far as HSCT-TMA pathophysiology and treatment are concerned.

Keywords

Complement, Complement gene mutations, Hematopoietic stem cell transplant (HSCT), Hemolytic uremic syndrome, Thrombotic microangiopathy (TMA)

Abbreviations:

aHUS, atypical hemolytic uremic syndrome

CFH, complement factor H

HSCT, hematopoietic stem cell transplant

MLPA, Multiplex Ligation-dependent Probe Amplification

NGS, next-generation sequencing

TMA, thrombotic microangiopathy

CRP, complement regulatory protein

AMD, Age-related macular degeneration

C3G, C3 glomerulopathy

Introduction

Hematopoietic stem cell transplant-related thrombotic microangiopathy (HSCT-TMA) is a very severe disorder burdened by high case-fatality rate with an unclear pathophysiology (1). Herein, we share our unexpected, yet potentially important, observation concerning six patients with HSCT-TMA in whom variants of complement system regulatory proteins have been acquired from their bone marrow donors and might have induced HSCT-TMA through dysregulation of the recipient complement system.

Materials and Methods

Patients brought to our attention with HSCT-TMA are screened for all the known conditions responsible for TMA (Homocysteine, ADAMTS13 function, CFH autoantibodies, variants and macro-rearrangements in *CFH* and related, *CFI*, *CFB*, *CD46*, *C3*, *DGKe*, *THBD* genes and risk haplotypes: CFH-H3 and MCPggaac) by means of relevant laboratory investigations, next-generation sequencing (NGS) and Multiplex Ligation-dependent Probe Amplification analysis (MLPA). The diagnosis of TMA was based on the simultaneous evidence of platelets consumption (low platelet count with increased mean platelet volume), non immune-mediated hemolysis (anemia with schistocytes and/or low haptoglobin and/or increased lactic dehydrogenase) and signs of renal damage (reduced renal function and or severe proteinuria and microscopic hematuria).
Patients' DNA was obtained from buccal brushing and recently our genetic screening has been extended to the donor's DNA obtained from recipient's peripheral blood cells.

Results and Discussion

In 6 out of 16 screened cases (all Caucasian but one Chinese and one African), specific genetic variants that were not present in the patient's DNA were identified in peripheral blood by NGS analysis (see table 1). MLPA analysis didn't show any pathogenic macro-rearrangements, whereas

homozygous CFH-H3 risk haplotype has been identified in patient #1 donor's DNA and both in donor's and recipient's DNA of patient #6 (2).

The potential pathogenetic role of complement dysregulation in HSCT-TMA has been previously reported (3), however to the best of our knowledge, the possibility that a mutation responsible for atypical hemolytic uremic syndrome (aHUS) may be "transplanted" with HSCT and expressed in the recipient has never been hypothesized and consequently this is not even regularly investigated.

The liver is considered the main source of circulating proteins of both the complement system and its soluble regulators, including CFH and related binding proteins, so that liver transplantation has been suggested in patients with aHUS due to CFH mutations. However, other hepatic and extrahepatic cells are known to produce this family of proteins, among which bone marrow-derived monocytes/macrophages in the peripheral blood and reticulo-endothelial system, including Kupffer cells (4). In an animal-model, Bejar postulated a possible role of Kupffer cells in increasing the risk of cardiovascular diseases following HSCT. He observes that homing of bone marrow donor derived-macrophages/Kupffer cells to the liver induced changes in lipid metabolism-related gene expression, thus transferring cardiovascular risk factors to the recipient (5). This is an indirect proof of liver colonization by donor-derived stem cells and consequent transfer of pathological risk factors.

Platelets are another source of CFH, which is already present on their surface upon exit from the bone marrow. Alexander demonstrated that wild type mice receiving a bone marrow transplant from their $Cfh^{-/-}$ counterparts undergo immune-complex disease due to an excessive accumulation in glomeruli (6).

A complementary observation made by Kiss (7) concerning a patient's remission from recurrent angioedema related to C1-inhibitor deficiency after HSCT suggests that the original genetic defect was possibly corrected by colonization of the recipient's liver by monocyte precursors, resulting in the production of sufficient amounts of the functional protein.

Considering all these independent observations, it is reasonable to hypothesize that in our patients the reticulo-endothelium has been "re-populated" by hematopoietic stem cell-derived elements carrying the donor's variant. The donor may not have experienced the disease given its low penetrance and because of the absence of triggers activating complement; whereas such triggers (chemotherapy, calcineurin inhibitors, transplant related cytokine storm, mucositis, infections, graft-versus-host disease, etc.) are all present in recipients during the weeks and months following HSCT. In all our cases, the TMA was relatively delayed with respect to HSCT (from 1 to 6 months) and this timing is compatible with the concept of reticulo-endothelial "re-population". Finally, we underline the response to anti-C5 inhibition in the two patients exposed to this treatment (#2 and #4), which further supports the hypothesis that the disease might have been related to complement dysregulation in keeping with previously reported positive experiences of other investigators (8-10).

A possible working hypothesis is that in our patients a donor-derived cell population (of the monocyte lineage) bearing a complement regulatory protein (CRP) variant, resulted in the production, and possibly systemic release, of a defective protein responsible for the impaired regulation of the native complement system. All variants detected in our series have already been described in patients with CRP disorders (see table references'), some demonstrating a significant association with aHUS, AMD or C3G, some others considered as predisposing genetic factors for aHUS or affecting the disease penetrance and severity.

Even though some genetic variants are presently not considered disease-causative by many experts since their functional pathogenic role has not always been demonstrated, nevertheless it is remarkable and worth being shared with the scientific community that the described patients exhibit a post-HSCT acquisition (from the donor) of uncommon gene changes involving the complement system.

A proof of concept would be the direct detection in blood of mutated proteins, which is currently out of reach for most laboratories.

Mechanisms linking mutations to TMA remain poorly understood and likely differ for individual proteins involved; reduced expression of the mutated allele (with low circulating levels of normal protein), as well as critical changes in affinity for binding substrates of mutated proteins may be at play.

Given the frequencies of the observed variants, the probability that they occur in combination with such a rare disease by chance in 6 of 16 patients is extremely low, making a pathogenic link between mutation and TMA plausible. We expect that extending the screening of both recipients and donors for complement gene mutations in patients with HSCT-TMA may allow the identification of additional cases and may also provide important insights into the pathophysiology of the condition itself.

Conflict of interest statement

The present study has not been published previously in whole or part. G.A. is a SAB member of the HUS Global Registry sponsored by Alexion Pharma INC. The other authors have no conflicts of interest to disclose.

Acknowledgments

The authors acknowledge Dr. Sonia Caccia (Dip. di Science Biomediche e Cliniche “Luigi Sacco”, Università di Milano, Italy), Martina Sgarbanti and Bice Strumbo (Laboratory of Medical Genetics, Fondazione IRCCS Ca’ Granda, Ospedale Maggiore Policlinico, Milano, Italy), Michela Perrone (Center for HUS Control, Prevention and Management, Fondazione IRCCS Ca’ Granda, Ospedale Maggiore Policlinico, Milano, Italy) for their technical assistance; Dr. Tommaso Mina and Dr. Nunzia Decembrino (Pediatric Hematology/Oncology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy) as well as Dr. Elisabetta Terruzzi (Divisione di Ematologia e CTA, Ospedale San Gerardo, Monza, Italy) for the clinical management of the patients; Dr. Dario Consonni (UO Epidemiologia, Dip. di Medicina Preventiva, Fondazione IRCCS Ca' Granda - Ospedale Maggiore

Policlinico, Milano, Italy) and Dr. Ivan Cortinovis (Laboratory G.A.Maccacaro, Dept. of Clinical Sciences and Community Health, University of Milan, Italy) for the statistical analysis.

References

1. Jodele S, Zhang K, Zou F et al. The genetic fingerprint of susceptibility for transplant-associated thrombotic microangiopathy. Blood. 2016;127(8):989–96. doi: 10.1182/blood-2015-08-663435.
2. Goodship TH, Cook HT, Fakhouri F, et al. Atypical hemolytic uremic syndrome and C3 glomerulopathy: conclusions from a "Kidney Disease: Improving Global Outcomes" (KDIGO) Controversies Conference. Kidney Int. 2017;91(3):539-551. doi:10.1016/j.kint.2016.10.005.
3. Jodele S, Licht C, Goebel J, et al. Abnormalities in the alternative pathway of complement in children with hematopoietic stem cell transplant-associated thrombotic microangiopathy. Blood. 2013;122(12):2003-7. doi: 10.1182/blood-2013-05-501445.
4. Schlaf G, Nitzki F, Heine I, Hardeland R, Schieferdecker HL, Götze O. C5a anaphylatoxin as a product of complement activation up-regulates the complement inhibitory factor H in rat Kupffer cells. Eur J Immunol. 2004 Nov;34(11):3257–66.
5. Bejar MT, Hernández-Vera R, Vilahur G, Badimon L. Bone Marrow Cell Transplant From Donors With Cardiovascular Risk Factors Increases the Pro-atherosclerotic Phenotype in the Recipients. Am J Transplant. 2016; Prepublished on Jul 16, 2016, as doi: 10.1111/ajt.13962.
6. Alexander JJ, Aneziokoro OGB, Chang A et al. Distinct and separable roles of the complement system in factor H-deficient bone marrow chimeric mice with immune complex disease. J Am Soc Nephrol JASN. 2006;17(5):1354–61.
7. Kiss TL, Messner HA, Galal A, Lipton J. Correction of recurrent angioedema related to C1 esterase inhibitor deficiency as a secondary event following nonmyeloablative allogeneic stem cell transplantation in a patient with myelofibrosis. Bone Marrow Transplant. 2005;35(2):213–4. doi: 10.1038/sj.bmt.1704748
8. Fernández C, Lario A, Forés R, Cabrera R. Eculizumab Treatment in a Patient with Hematopoietic Stem Cell Transplantation-Associated Thrombotic Microangiopathy and

Steroid-Refractory Acute Graft Versus Host Disease. Hematol Rep. 2015;7(4):6107. doi: 0.4081/hr.2015.6107.

9. Jodele S, Fukuda T, Vinks A, et al. Eculizumab therapy in children with severe hematopoietic stem cell transplantation-associated thrombotic microangiopathy. Biol Blood Marrow Transplant. 2014;20(4):518-25. doi: 10.1016/j.bbmt.2013.12.565.

10. de Fontbrune FS, Galambrun C, Sirvent A, et al. Use of Eculizumab in Patients With Allogeneic Stem Cell Transplant-Associated Thrombotic Microangiopathy: A Study From the SFGM-TC. Transplantation. 2015;99(9):1953-9. doi: 10 \azP.1097/TP.0000000000000601.

Table 1: patient's characteristics and genetic workout

àìCase	Age (yrs)	Primary Disease	Genetic Findings		MAF (ExAC)	Ref.
			Recipient's DNA	Donor's DNA		
#1	21	Burkitt Lymphoma	WT	CFH c.1548T>A (p.Asn516Lys) CFHR5 c.1067 G>A (p.Arg356His)	0.0004046 0.01777	1 2
#2	15	Blackfan Diamond Anemia	WT	CFHR5 c.485_486dupAA (p.Glu163Lysfs*10)	0.005892	3
#3	59	Non Hodgkin Lymphoma	WT	CFB c.724A>C (p.Ile242Leu)	Unknown	4
#4	55	Non Hodgkin Lymphoma	CFHR5 c.136C>T (p.Pro46Ser)	C3 c.463A>C (p.Lys155Gln)	0.003362 0.0059^	5 6
#5	56	ALL	WT	CFI c.1217G>A (p.Arg406His) MCP c.38C>T (p.Ser13Phe)	0.01688 0.005003	7 8
#6	13	Sickle-cell Disease	WT	CFI c.1246A>C (p.Ile416Leu) C3 c.4645C>A (p.Leu1549Met) CFHR5 c.1704T>A (p.Cys568*) CFHR3 c.299A>G (p.Tyr100Cys)	0.001113 0.001164 0.002508 0.001036	8 - - -

Legend: WT: wild type; MAF (Minor Allele Frequency); ^:MAF referred to recipient's variant; ALL: acute lymphoblastic leukemia; ExAC: http://exac.broadinstitute.org/

References to table 1:

1. Le Quintrec M, Lionet A, Kamar N, et al. Complement mutation-associated de novo thrombotic microangiopathy following kidney transplantation. Am J Transplant. 2008;8(8):1694–701. doi:10.1111/j.1600-6143.2008.02297.x

2. Monteferrante G, Brioschi S, Caprioli J, et al. Genetic analysis of the complement factor H related 5 gene in haemolytic uraemic syndrome. Mol Immunol. 2007;44(7):1704-8. doi:10.1016/j.molimm.2006.08.004

3. Vernon KA, Goicoechea de Jorge E, Hall AE, et al. Acute presentation and persistent glomerulonephritis following streptococcal infection in a patient with heterozygous complement

factor H-related protein 5 deficiency. Am J Kidney Dis. 2012;60(1):121–5. doi:10.1053/j.ajkd.2012.02.329

4. Marinozzi MC, Vergoz L, Rybkine T, et al. Complement factor B mutations in atypical hemolytic uremic syndrome-disease-relevant or benign? J Am Soc Nephrol. 2014;25(9):2053-65. doi:10.1681/ASN.2013070796

5. Helgason H, Sulem P, Duvvari MR, et al. A rare nonsynonymous sequence variant in C3 is associated with high risk of age-related macular degeneration. Nat Genet. 2013;45(11):1371–4. doi: 10.1038/ng.2740

6. Skerka C, Chen Q, Fremeaux-Bacchi V, Roumenina LT. Complement factor H related proteins (CFHRs). Mol Immunol. 2013;56(3):170-80. doi:10.1016/j.molimm.2013.06.001

7. Fremeaux-Bacchi V, Dragon-Durey M-A, Blouin J, et al. Complement factor I: a susceptibility gene for atypical haemolytic uraemic syndrome. J Med Genet. 2004;41(6):e84.

8. Rodríguez de Córdoba S, Hidalgo MS, Pinto S, Tortajada A. Genetics of atypical hemolytic uremic syndrome (aHUS). Semin Thromb Hemost. 2014;40(4):422-30. doi: 10.1055/s-0034-1375296