the literature and meta-analysis. *Clin Lymphoma Myeloma Leuk*. 2013;13(4):424-429.

2. Delforge M, Ludwig H. How I manage the toxicities of myeloma drugs. *Blood*. 2017;129(17):2359-2367.

3. Sviggum HP, Davis MDP, Rajkumar SV, Dispenzieri A. Dermatologic adverse effects of lenalidomide therapy for amyloidosis and multiple myeloma. *Arch Dermatol*. 2006;142(10):1298-1302.

4. Benjamin M, Reddy S, Brawley OW. Myeloma and race: a review of the literature. *Cancer Metastasis Rev*. 2003;22(1):87-93.

5. Benboubker L, Dimopoulos MA, Dispenzieri A, et al; FIRST Trial Team. Lenalidomide and dexamethasone in transplant-ineligible patients with myeloma. *N Engl J Med*. 2014;371(10):906-917.

DOI 10.1182/blood.2021010853

TO THE EDITOR:

Markers of complement activation in plasma during quiescent phases in patients with catastrophic antiphospholipid syndrome

Amelia Ruffatti,[1] Marta Tonello,[2] Paolo Macor,[3] Antonia Calligaro,[2] Teresa Del Ross,[2] Maria Favaro,[2] Virginia Lotti,[4] Antonio Carletto,[4] Ariela Hoxha,[5] and Domenico Biasi[4]

[1]Department of Medicine and [2]Rheumatology Unit, Department of Medicine, University Hospital of Padua, Padua, Italy; [3]Department of Life Sciences, University of Trieste, Trieste, Italy; [4]Rheumatology Unit, Department of Medicine, University Laboratories for Medical Research (LURM) University Hospital of Verona, Verona, Italy; and [5]Internal Medicine Unit, Department of Medicine, San Bortolo Hospital, Vicenza, Italy

Complement activation has been implicated in the pathogenesis of catastrophic antiphospholipid syndrome (CAPS), a rare life-threatening disease.[1-5] Low C3 and C4 levels that have been identified in patients during acute or relapsing CAPS have, in fact, been considered signs of complement activation and consumption.[1-3] Likewise, complement activation products and high levels of C5b-9 terminal complex have been detected in the plasma of patients with acute CAPS.[2,4] Studies by Chaturvedi et al have further broadened our understanding of the complement system's role in the pathogenesis of CAPS thrombotic storm by demonstrating that sera from acute phase patients induced complement-dependent cell killing and cell-surface deposition of C5b-9 on *PIGA*null TF-1 cells.[5] No signs of complement activation during quiescent CAPS phases, that is, long before its onset or after its remission, have ever been reported. High C5b-9 levels (1657 U; normal value <200) were recently identified in a patient with CAPS that had severe cardiac involvement immediately before treatment was begun.[4] She was refractory to the so-called triple therapy (anticoagulation + steroids + plasma exchange/IV immunoglobulins)[6] and was successfully treated with eculizumab. Unexpectedly, 5 months after a complete recovery (eculizumab had been suspended 3 months earlier), the patient's C5b-9 plasma levels were found to be similar (1804 U) to those registered during the acute phase.[4] In the current study, plasma levels of C5b-9 terminal complex and C5a anaphylatoxin during the quiescent phases of CAPS were investigated, compared with control populations, and discussed.

The plasma of 7 patients who had suffered from CAPS between 2009 and 2017 was collected during the quiescent CAPS phases, 3 before (median, 57 months; interquartile range [IQR], 26) and 4 after (median, 38 months; IQR, 24.2) the acute CAPS episode. The samples were stored at −80°C. Plasma concentrations of C5b-9 and C5a were assessed using the MicroVue C5b-9 Plus EIA and the MicroVue C5a Plus EIA, respectively (QUIDEL, San Diego, CA). There were 6 women and 1 man with a median age of 44 years (IQR, 20). Because histological studies were performed only in 2 cases, 2 patients had definite, and 5 had probable CAPS, according to Asherson et al classification criteria.[7] Eight patients with antiphospholipid syndrome (APS) who had experienced a thrombosis over the preceding year and 8 healthy subjects sex and age matched with the quiescent patients with CAPS were used as the control populations. The median follow-up of the APS patients was 15 years (IQR, 9.2). The study was approved by the regional ethics committee and was carried out in accordance with the 1964 Declaration of Helsinki. Written informed consent was obtained from all the participants.

The patients' demographic and clinical characteristics along with the time to recovery and antiphospholipid antibody profile at time of diagnosis are outlined in Table 1. All were successfully treated using conventional triple therapy, and none required therapeutic complement inhibition. The results of C5b-9 and C5a analyses during the quiescent phases in the patients with CAPS and in the controls are outlined in Figure 1. The C5b-9 levels in the quiescent CAPS were significantly higher than those in the thrombotic patients with APS (median, 330.2 ng/mL; IQR, 86.6; vs median, 189.7 ng/mL; IQR, 138.9) and in the healthy controls (median, 330.2 ng/mL; IQR, 86.6; vs median, 120.9 ng/mL; IQR, 83.7). Similarly, the C5a levels were significantly higher in the quiescent patients with CAPS with respect to the thrombotic patients with APS (median, 27.6 ng/mL; IQR, 58.4; vs median, 8.9 ng/mL; IQR, 8.3) and the healthy controls (median, 27.6 ng/mL; IQR, 58.4; vs median, 4.9 ng/mL; IQR, 4.9).

There are some investigations in the literature reporting elevated plasma levels of C5b-9[2,4,8,9] and C5a[9] in the active phase of CAPS[2,4] or thrombotic APS.[8,9] The current study demonstrates for the first time the finding of significantly high C5b-9 and C5a plasma levels during the quiescent phases long before the onset of the acute phase and after CAPS remission. Excessive delivery of complement activation products in the plasma of quiescent patients with CAPS both before and after an acute episode

Table 1. Demographic and clinical characteristics of the study population

Age/ sex	Antiphospholipid antibody profile	Previous diagnosis	CAPS involvement	Treatment	Outcome (time to recovery)
50/F	IgG aCL 77 GPL IgM aCL negative IgG aβ2GPI 102 U IgM aβ2GPI negative LAC positive	Thrombotic + obstetric PAPS	Kidney, heart, skin, lung	AC + PE + S + IVIG + low-dose aspirin	Complete recovery (20 d)
35/F	IgG aCL 118 GPL IgM aCL negative IgG aβ2GPI 326 U IgM aβ2GPI negative LAC positive	Thrombotic + obstetric PAPS	Skin, liver, lung, right adrenal gland	AC + PE + S + IVIG + low-dose aspirin	Complete recovery (8 d)
29/M	IgG aCL 95 GPL IgM aCL 61 MPL IgG aβ2GPI 101 U IgM aβ2GPI 43 U LAC positive	Antiphospholipid antibody carrier	Brain, kidney, heart, retina, skin	AC + PE + S + IVIG + low-dose aspirin	Complete recovery (10 d)
56/F	IgG aCL 100 GPL IgM aCL negative IgG aβ2GPI 21 U IgM aβ2GPI negative LAC positive	Previous CAPS	Colon, lung, heart	AC + PE + S + IVIG + double anti-platelet therapy	Complete recovery (8 d)
44/F	IgG aCL 30 GPL IgM aCL negative IgG aβ2GPI27 U IgM aβ2GPI negative LAC positive	Thrombotic APS + SLE	Lung, liver, inferior mesenteric artery, infrarenal aorta	AC + PE + S + IVIG	Partial recovery (10 d) Large vessel thrombosis stabilized outcomes
47/F	IgG aCL 69 GPL IgM aCL88 MPL IgG aβ2GPI 101 U IgM aβ2GPI 39 U LAC positive	Thrombotic APS + lupuslike	Kidney, ischemic cholecystitis, left adrenal gland	AC + PE + S	Partial recovery (8 d) Renal failure GFR: 50 mL/min
30/F	IgG aCL 101 GPL IgM aCL 131 MPL IgG aβ2GPI 154 U IgM aβ2GPI 80 U LAC positive	Thrombotic APS	Heart, lung, skin	AC + PE + S + IVIG + low-dose aspirin	Partial recovery (16 d) Cardiomyopathy NYHA class I to II

AC, anticoagulant drugs; F, female; GFR, glomerular filtration rate; GPL, G phospholipid units; IgG aCL, immunoglobulin G anticardiolipin; IgM aCL, immunoglobulin M anticardiolipin; IgG aβ2GPI, immunoglobulin G anti-β2 glycoprotein I; IgM aβ2GPI, immunoglobulin M anti-β2 glycoprotein I; IVIG, IV immunoglobulins; LAC, lupus anticoagulant; M, male; MPL, M phospholipid units; NYHA, New York Heart Association; PAPS, primary antiphospholipid syndrome; PE, plasma exchange; S, steroids; SLE, systemic lupus erythematosus.

suggests that there may be a defective complement control in these patients. Chaturvedi et al, who identified a high prevalence of rare germline variants in complement regulatory genes in patients with CAPS, speculated that in the presence of antiphospholipid antibodies, patients who also have a pathogenic complement regulatory gene mutation are predisposed to uncontrolled activation of complement leading to CAPS when a trigger factor emerges. No functional data concerning the pathogenic role of the rare germline variants in CAPS were however provided by this study.[5] The significantly higher C5b-9 and C5a plasma levels during the quiescent phases could be a feature of patients with APS that have a predisposition to developing CAPS in the presence of a precipitating factor. The absence of CAPS over a long follow-up period (median, 15 years) in patients with thrombotic APS and the occurrence of relapses, at times even repeated ones, in patients who had already experienced a CAPS episode seem to confirm a predisposition to the disorder.[10] The low number of patients with CAPS available for our study is one of the study's limits that can be explained and justified by the rarity of the disease. Furthermore, we did not have serial samples from our patients with quiescent CAPS to draw solid conclusions regarding the persistence of elevated levels of C5b-9 and C5a over time both before and after the CAPS episode and their clinical significance. If our data are confirmed by multicenter studies investigating large CAPS and control populations and repeated samples from each quiescent patient with CAPS, high C5b-9 and C5a levels in patients with APS will be able identify subjects at risk of CAPS who would benefit from complement inhibitor therapy during a CAPS episode.

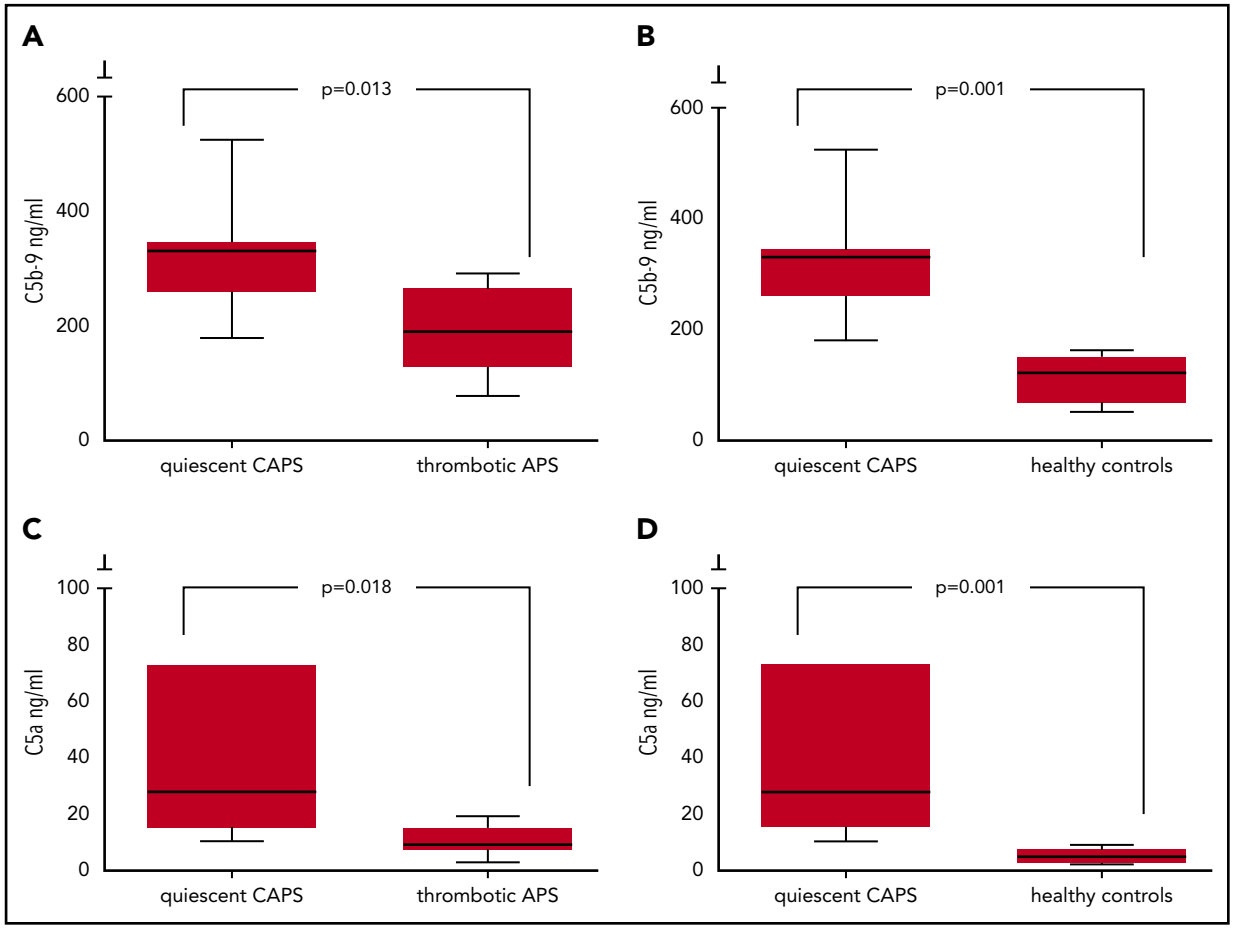

Figure 1. C5b-9 and C5a plasma levels are significantly higher in the patients during the quiescent phases of CAPS than in the thrombotic patients with APS and healthy controls. (A) Results of statistical comparison of C5b-9 plasma levels between the quiescent patients with CAPS and the thrombotic patients with APS, and (B) between the quiescent patients with CAPS and the healthy controls. (C) Results of statistical comparison of C5a plasma levels between the quiescent patients with CAPS and the thrombotic patients with APS, and (D) between the quiescent patients with CAPS and the healthy controls.

Currently, eculizumab is the most available anticomplement drug; it is a humanized monoclonal antibody targeted against complement C5 that inhibits its cleavage and prevents the generation of prothrombotic and proinflammatory molecules and membrane attack complex C5b-9. Although 12 case reports investigating its use to treat CAPS have been published,[1-4,11-18] there are yet no guidelines for its timing of administration and appropriate dosage in CAPS. Given the rapid clinical improvement noted already at the time of the third injection in the majority of CAPS patients, the dosage used during the induction phase seems to be crucial.[11] Although the most common induction dose, which was 900 mg/wk, produced both a good response[12,14,16,17] and a partial remission,[1,11,15] beginning treatment immediately or within 9 days of a CAPS diagnosis was associated with a better outcome[2-4,12,17] with respect to a later start.[1,11,15] The recent report by Yelnik et al represents the first cohort study on the use of eculizumab in CAPS and describes the results obtained in 11 patients.[19] Five of them (45.4%) only had a significant improvement in the few days after the first administration. Unfortunately, in this study, eculizumab was often used in the presence of severe and prolonged organ failures indicative of irreversible injury. There was, in fact, more patients who required permanent hemodialysis prior to receiving eculizumab in the nonresponders with respect to the responders.[19] The efficacy and safety of eculizumab in CAPS remain to be clarify by well-designed multicenter studies that take into account the rapid evolution of CAPS in organ failure and the importance of using eculizumab in the early stages of the disease.[6,20]

Authorship

Contribution: A.R. developed the project, analyzed the data, and wrote the manuscript; M.T. performed laboratory assays and collected and analyzed the data; P.M. analyzed the data and reviewed the paper; A. Calligaro, T.D.R., M.F., A. Carletto, and A.H. monitored the CAPS and APS patients and critically reviewed the paper; V.L. performed laboratory assays and collected the data; and D.B. contributed to data collection and analysis and critically reviewed the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

ORCID profiles: A.R., 0000-0003-1391-9828; P.M., 0000-0003-3079-4019; A. Carletto, 0000-0002-3350-4438; A.H., 0000-0001-7741-6432; D.B., 0000-0001-8055-844X.

Correspondence: Amelia Ruffatti, Department of Medicine, Palasanità, Via Giustiniani, 2-35128 Padova, Italy; e-mail: amelia.ruffatti@unipd.it.

Footnotes

Submitted 28 December 2020; accepted 5 February 2021; prepublished online on *Blood* First Edition 1 March 2021.

Please e-mail the corresponding author for original data. Although the idea for the study originated from observation on our recently published case report (listed in Ruffatti et al[4]), that patient was not enrolled in the current investigation.

REFERENCES

1. Kronbichler A, Frank R, Kirschfink M, et al. Efficacy of eculizumab in a patient with immunoadsorption-dependent catastrophic antiphospholipid syndrome: a case report. *Medicine (Baltimore)*. 2014;93(26):e143.
2. Barratt-Due A, Fløisand Y, Orrem HL, et al. Complement activation is a crucial pathogenic factor in catastrophic antiphospholipid syndrome. *Rheumatology (Oxford)*. 2016;55(7):1337-1339.
3. Tinti MG, Carnevale V, Inglese M, et al. Eculizumab in refractory catastrophic antiphospholipid syndrome: a case report and systematic review of the literature. *Clin Exp Med*. 2019;19(3):281-288.
4. Ruffatti A, Tarzia V, Fedrigo M, et al. Evidence of complement activation in the thrombotic small vessels of a patient with catastrophic antiphospholipid syndrome treated with eculizumab. *Autoimmun Rev*. 2019;18(5):561-563.
5. Chaturvedi S, Braunstein EM, Yuan X, et al. Complement activity and complement regulatory gene mutations are associated with thrombosis in APS and CAPS. *Blood*. 2020;135(4):239-251.
6. Rodriguez-Pintó I, Espinosa G, Cervera R. Catastrophic antiphospholipid syndrome: The current management approach. *Best Pract Res Clin Rheumatol*. 2016;30(2):239-249.
7. Asherson RA, Cervera R, de Groot PG, et al; Catastrophic Antiphospholipid Syndrome Registry Project Group. Catastrophic antiphospholipid syndrome: international consensus statement on classification criteria and treatment guidelines. *Lupus*. 2003;12(7):530-534.
8. Davis WD, Brey RL. Antiphospholipid antibodies and complement activation in patients with cerebral ischemia. *Clin Exp Rheumatol*. 1992;10(5):455-460.
9. Meroni PL, Macor P, Durigutto P, et al. Complement activation in antiphospholipid syndrome and its inhibition to prevent rethrombosis after arterial surgery. *Blood*. 2016;127(3):365-367.
10. Espinosa G, Rodríguez-Pintó I, Gomez-Puerta JA, Pons-Estel G, Cervera R; Catastrophic Antiphospholipid Syndrome (CAPS) Registry Project Group (European Forum on Antiphospholipid Antibodies). Relapsing catastrophic antiphospholipid syndrome potential role of microangiopathic hemolytic anemia in disease relapses. *Semin Arthritis Rheum*. 2013;42(4):417-423.
11. Skoczynska M, Crowther MA, Chowaniec M, Ponikowska M, Chaturvedi S, Legault K. Thrombotic microangiopathy in the course of catastrophic antiphospholipid syndrome successfully treated with eculizumab: case report and systematic review of the literature. *Lupus*. 2020;29(6):631-639.
12. Shapira I, Andrade D, Allen SL, Salmon JE. Brief report: induction of sustained remission in recurrent catastrophic antiphospholipid syndrome via inhibition of terminal complement with eculizumab. *Arthritis Rheum*. 2012;64(8):2719-2723.
13. Silva RA, Moshfeghi DM. Antiphospholipid antibody-associated choroidopathy. *Eye (Lond)*. 2014;28(6):773-774.
14. Strakhan M, Hurtado-Sbordoni M, Galeas N, Bakirhan K, Alexis K, Elrafei T. 36-year-old female with catastrophic antiphospholipid syndrome treated with eculizumab: a case report and review of literature. *Case Rep Hematol*. 2014;2014:704371.
15. Zikos TA, Sokolove J, Ahuja N, Berube C. Eculizumab induces sustained remission in a patient with refractory primary catastrophic antiphospholipid syndrome. *J Clin Rheumatol*. 2015;21(6):311-313.
16. Wig S, Chan M, Thachil J, Bruce I, Barnes T. A case of relapsing and refractory catastrophic anti-phospholipid syndrome successfully managed with eculizumab, a complement 5 inhibitor. *Rheumatology (Oxford)*. 2016;55(2):382-384.
17. Guillot M, Rafat C, Buob D, et al. Eculizumab for catastrophic antiphospholipid syndrome-a case report and literature review. *Rheumatology (Oxford)*. 2018;57(11):2055-2057.
18. Kello N, Khoury LE, Marder G, Furie R, Zapantis E, Horowitz DL. Secondary thrombotic microangiopathy in systemic lupus erythematosus and antiphospholipid syndrome, the role of complement and use of eculizumab: Case series and review of literature. *Semin Arthritis Rheum*. 2019;49(1):74-83.
19. Yelnik CM, Miranda S, Mékinian A, et al. Patients with refractory catastrophic antiphospholipid syndrome respond inconsistently to eculizumab. *Blood*. 2020;136(21):2473-2477.
20. Ruffatti A, De Silvestro G, Marson P, et al. Catastrophic antiphospholipid syndrome: Lessons from 14 cases successfully treated in a single center. A narrative report. *J Autoimmun*. 2018;93:124-130.

DOI 10.1182/blood.2020010575

TO THE EDITOR:

Clonal hematopoiesis and therapy-related myeloid neoplasms following neuroblastoma treatment

Tim H. H. Coorens,[1,*] Grace Collord,[1-3,*] Wanhua Lu,[4] Emily Mitchell,[1,4,5] Jannat Ijaz,[1] Thomas Roberts,[4] Thomas R. W. Oliver,[1] G. A. Amos Burke,[4] Michael Gattens,[4] Emmy Dickens,[4] Jyoti Nangalia,[1,4-6] Marc Tischkowitz,[7] John Anderson,[8,9] Adam Shlien,[10] Anna L. Godfrey,[4] Matthew J. Murray,[4,11,†] and Sam Behjati[1,4,12,†]

[1]Wellcome Sanger Institute, Hinxton, United Kingdom; [2]Department of Haematology, University College London Hospital, London, United Kingdom; [3]Department of Haematology, University College London Cancer Institute, London, United Kingdom; [4]Cambridge University Hospitals National Health Service (NHS) Foundation Trust, Cambridge, United Kingdom; [5]Department of Haematology, University of Cambridge, Cambridge, United Kingdom; [6]Cambridge Stem Cell Institute, Cambridge, Cambridgeshire, United Kingdom; [7]Department of Medical Genetics, National Institute for Health Research Cambridge Biomedical Research Centre, University of Cambridge, Cambridge, United Kingdom; [8]University College London Great Ormond Street Institute of Child Health, London, United Kingdom; [9]Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom; [10]The Hospital for Sick Children, Toronto, ON, Canada; and [11]Department of Pathology and [12]Department of Paediatrics, University of Cambridge, Cambridge, United Kingdom

Therapy-related myeloid neoplasms (TMNs) constitute one of the most challenging complications of cancer treatment.[1] Although understanding of the pathogenesis of TMNs remains fragmentary, genomic studies in adults have thus far refuted the notion that TMNs simply result from cytotoxin-induced DNA damage.[2-4] Analysis of the preclinical evolution of a limited number of adult TMNs have traced the majority of cases to clonal hematopoiesis (CH) that predates cytotoxic treatment and lacks the mutational footprint of genotoxic therapies.[2-6] Balanced translocations, generally attributed to treatment with topoisomerase II