Consumption of complement in a 26-year-old woman with severe thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination

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ABSTRACT

Extremely rare reactions characterized by thrombosis and thrombocytopenia have been described in subjects that received ChAdOx1 nCoV-19 vaccination 5–16 days earlier. Although patients with vaccine-induced thrombotic thrombocytopenia (VITT) have high levels of antibodies to platelet factor 4 (PF4)–polyanion complexes, the exact mechanism of the development of thrombosis is still unknown. Here we reported serum studies as well as proteomics and genomics analyses demonstrating a massive complement activation potentially linked to the presence of anti-PF4 antibodies in a patient with severe VITT. At admission, complement activity of the classical and lectin pathways were absent (0% for both) with normal levels of the alternative pathway (73%) in association with elevated levels of the complement activation marker sC5b-9 (630 ng/mL [n.v. 139–462 ng/mL]) and anti-PF4 IgG (1.918 OD [n.v. 0.136–0.300 OD]). The immunoblotting analysis of C2 showed the complete disappearance of its normal band at 110 kDa. Intravenous immunoglobulin treatment allowed to recover complement activity of the classical pathway (91%) and lectin pathway (115%), to reduce levels of sC5b-9 (135 ng/mL) and anti-PF4 IgG (0.681 OD) and to normalize the C2 pattern at immunoblotting. Proteomics and genomics analyses in addition to serum studies showed that the absence of complement activity during VITT was not linked to alterations of the C2 gene but rather to a strong complement activation leading to C2 consumption. Our data in a single patient suggest monitoring complement parameters in other VITT patients considering also the possibility to target complement activation with specific drugs.

1. Introduction

Vaccine-induced thrombotic thrombocytopenia (VITT) is an extremely rare condition that may develop after vaccination with the recombinant adenoviral vector encoding the spike protein antigen of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (ChAdOx1 nCov-19, AstraZeneca) [1]. At the beginning, it was observed in 1 case per 100,000 exposures; however, at present some additional cases have been reported [2]. Thrombotic events, which begin 5–16 days after vaccination, mainly include cerebral venous thrombosis, splanchnic vein thrombosis and pulmonary embolism [1]. Low platelet counts, high levels of D-dimer and low fibrinogen have been described at

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diagnosis. Another distinctive characteristic of these patients is the presence of antibodies to platelet factor 4 (PF4)–polyanion complexes. Treatment options consist of high-dose intravenous immunoglobulin (IVIG) and the same anticoagulants used to treat heparin-induced thrombocytopenia, i.e. direct Xa- or thrombin-inhibitors or fondaparinux [1]. Ischemic brain injury, superimposed hemorrhage, or both conditions, often after anticoagulation may concur to mortality in some cases [2].

To date, several questions remain open concerning the components of the vaccine that are implicated in the adverse event as well as the risk factors of the subjects and the mechanisms that lead to the coagulation derangement. Since on theoretical basis complement activation has been proposed as a link between PF4-containing immune complexes and thrombosis [3] we evaluated the involvement of the complement system in a patient with VITT that occurred after ChAdOx1 nCov-19 vaccination.

2. Methods

2.1. Functional immuno-enzymatic assay for the classical, alternative and mannose-binding lectin complement pathways

The functional tests for the classical, alternative and mannose-binding lectin (MBL) pathways were performed using the Wieslab Complement System kit (Euro-Diagnostica, Malmö, Sweden), following the manufacturer's instructions. The wells of the microtiter strips are coated with a specific activator of the tested complement pathway, and serum samples are diluted in a buffer containing specific blockers of the other two complement pathways in order to ensure that only the tested pathway is activated during incubation. The wells are then washed, and C5b-9 is detected with a specific alkaline phosphatase-labeled antibody against the neoantigen expressed on C9 during C5b-9 formation. After a further washing step, the specific antibodies are detected by means of incubation with the alkaline phosphatase substrate solution. Results are expressed as percentages of the activity of a standard sample (i.e., normal pooled serum fixed at 100%) [4].

2.2. Hemolytic assay for functional activity of the classical complement pathway

Functional activity of complement was obtained measuring complement hemolytic activity 50% (CH50) on sensitized sheep erythrocytes as previously described [5].

2.3. Visualization and quantification of C2

Proteins in serum samples were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After electrophoretic transfer of proteins from gel onto a polyvinylidene difluoride membrane (Immobilon; Millipore, Bedford, Mass.), the presence of C2 was documented using a biotin-conjugated goat anti-human C2 (Quidel, San Diego, CA) and phosphatase-labeled streptavidin (Sigma-Aldrich, Milan, Italy).

C2 concentration in serum samples was measured by an ELISA, in which polystyrene plates (Thermo Fisher Scientific, Milan, Italy) were coated overnight with goat anti-human C2 (Quidel, 10 μg/mL) and revealed by biotin-conjugated anti-C2 polyclonal antibody (Sigma-Aldrich). The interaction of the biotin-conjugated antibodies anti-C2 with non-conjugated anti-C2 antibodies was excluded by testing several dilutions.

2.4. Measurement of anti-C2 antibodies and immune complexes

Two specific ELISA were developed. In both cases, goat anti-C2 antibodies (Quidel) were bound on polystyrene plate (Thermo Fisher Scientific), washed and plate blocked using 2% BSA in PBS. a) Diluted serum samples were incubated and the presence of C2/anti-C2 immune complexes was analyzed adding an alkaline phosphatase-conjugated goat anti-human IgG (Sigma-Aldrich) and then pNPP (Sigma-Aldrich). b) Coated and blocked plates were also incubated first with NHS (as a source of C2) and then with serum samples; the presence of anti-C2 antibodies was evaluated again adding an alkaline phosphatase-conjugated goat anti-human IgG (Sigma-Aldrich) and then pNPP (Sigma-Aldrich). The plates were read at 405 nm using a Titertek Multiskan ELISA reader (Flow Labs). The interaction of the secondary antibodies with non-conjugated anti-C2 antibodies was excluded by testing several dilutions.

2.5. Purification of C2

Purification of C2 from serum samples was performed by affinity chromatography. Purified anti-C2 polyclonal antibodies (1 mg, Quidel) were bound on activated BrCN-sepharose following the instruction of the company (Sigma-Aldrich), and equally divided in 3 tubes for the incubation with serum samples. After 4 h at 4 °C, tubes were centrifuges, supernatants were eliminated and C2 obtained adding Sodium Citrate at pH 3.

2.6. Anti-platelet factor 4 (PF4) autoantibodies

The presence of anti-PF4 IgG antibodies levels were evaluated by the PF4 IgG solid-phase ELISA test (Immucor, Milan, Italy) in which the microwells are coated with PF4 complexed to polyvinyl sulfonate. The coefficient of variation of the test was 10%. Optical density values $\geq$ 0.400 were considered positive (specificity 100%, sensitivity 60%).

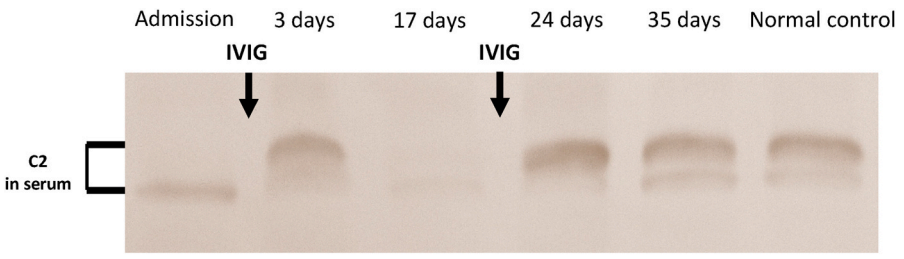
2.7. Sample preparation for proteomic analysis

In order to improve the identification and quantification of plasma proteins we depleted high-abundant proteins using the Seppro IgY14 spin column kit (Sigma-Aldrich Inc., St. Louis, MO, USA) according to the manufacturer's procedure. The method was used to bind human serum HSA, IgG, fibrinogen, transferrin, IgA, IgM, haptoglobin, alpha 2-macroglobulin, alpha 1-acid glycoprotein, alpha 1-antitrypsin, Apo A-I HDL, Apo A-II HDL, complement C3 and LDL (ApoB) in order to increase low-abundance protein identification. The samples were transferred into an Amicon Ultra-0.5 mL 3 kDa centrifugal filter (Millipore, Billerica, MA, USA) following the manufacturer's procedure to collect high molecular weight proteins.

The immunodepleted plasma samples and the immune-precipitated complement C2 samples were then subjected to reduction with DTT 200 mM, to alkylation with IAM 200 mM and to complete protein digestion with 2 μg of Trypsin (Sigma-Aldrich Inc., St. Louis, MO, USA). The peptide digests were desalted on the Discovery® DSC-18 solid phase extraction (SPE) 96-well plate (25 mg/well) (Sigma-Aldrich Inc., St. Louis, MO, USA). The SPE plate was preconditioned with 1 mL of acetonitrile and 2 mL of water. After loading the sample, the SPE was washed with 1 mL of water. The adsorbed proteins were eluted with 800 μL of acetonitrile:water (80:20). After the desalting process, the sample was vacuum-evaporated and reconstituted in mobile phase for the analysis.

2.8. Proteomics analysis and data processing

The digested peptides were analyzed with a UHPLC Vanquish system (Thermo Scientific, Rodano, Italy) coupled with an Orbitrap Q-Exactive Plus (Thermo Scientific, Rodano, Italy). Peptides were separated by a reverse phase column (Accucore™ RP-MS 100 × 2.1 mm, particle size 2.6 μm). The column was maintained at a constant temperature of 40 °C at a flow rate of 0.200 mL/min. Mobile phase A and B were water and acetonitrile respectively, both acidified with 0.1% formic acid. The analysis was performed using the following gradient: 0–5 min from 2%



Complement classical pathway activity (%)	0	78	0	91	102	[69-129]
Complement MBL pathway activity (%)	0	103	1	115	116	[0-125]
Complement alternative pathway activity (%)	73	76	80	96	135	[30-113]
CH50 (U/ml)	0	862	0	864	899	[900-1300]
C2 antigen (μg/ml)	59.8	31.6	69.6	34.8	58.0	[50-100]
SC5b-9 (ng/ml)	630	155	553	135	136	[139-462]
Anti-PF4 autoantibody (OD)	1.918	0.897	2.228	0.681	0.455	[0.136-0.300]
Total protein (mg/ml)	93.7	128.3	86.4	108.7	91.4	[55-95]

Fig. 1. Complement analysis in serum and plasma. Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting analysis of C2 at admission and at different time points after infusion of intravenous immunoglobulin (IVIG). The last lane refers to normal controls. Circulating levels of complement parameters, anti-platelet factor 4 (PF4) and total proteins as well as their normal ranges are also reported.

to 5% B; 5–55 min from 5% to 30% B; 55–61 from 30% to 90% B and hold for 1 min, at 62.1 min the percentage of B was set to the initial condition of the run at 2% and hold for about 8 min in order to rebalance the column, for a total run time of 70 min. The Mass spectrometry analysis was performed in positive ion mode. The ESI source was used with a voltage of 2.8 kV. The capillary temperature, sheath gas flow, auxiliary gas and spare gas flow were set at 325 °C, 45 arb, 10 arb and 2 respectively. S-lens was set at 70 rf. For the acquisition of spectra a data-dependent (ddMS2) top 10 scan mode was used. Survey full-scan MS spectra (mass range m/z 381 to 1581) were acquired with resolution R = 70,000 and AGC target 3×10^6. MS/MS fragmentation was performed using high-energy c-trap dissociation (HCD) with resolution R = 35,000 and AGC target 1×10^6. The normalized collision energy (NCE) was set to 30. The injection volume was 3 μl.

The mass spectra analysis was carried out using MaxQuant software (version 1.6.14) and Mascot (Matrix Science Inc., Boston, USA). MaxQuant parameters were set as follow: trypsin was selected for enzyme specificity; the search parameters were fixed to an initial precursor ion tolerance of 10 ppm and MS/MS tolerance at 20 ppm; as fixed modification, carbamidomethylation was set, whereas oxidation was set as variable modification. The maximum missed cleavages were set to 2. Andromeda search engine searched the spectra in MaxQuant against the Uniprot_CP_Human_2018 sequence database. Label free quantification was performed including a match between runs option with the following parameters: protein and peptide false discovery rate was set to 0.01; the quantification was based on the extracted ion chromatograms, with a minimum ratio count of 1; the minimum required peptide length was set to 7 amino acids. The Mascot search was performed on Mascot v. 2.4, the digestion enzyme selected was trypsin, with 2 missed cleavages and a search tolerance of 10 ppm was specified for the peptide mass tolerance, and 0.1 Da for the MS/MS tolerance. The charges of the peptides to search for were set to 2 +, 3 + and 4 +, and the search was set on monoisotopic mass. The following modifications were specified for the search: carbamidomethyl cysteines as fixed modification and oxidized methionine as variable modification [6]. Statistical analyses were done using GraphPad Prism v. 8 and GraphRobot (https://www.graphrobot.com/).

2.9. Clinical exome sequencing (CES)

Following written informed consent, genomic DNA of the proband was extracted from peripheral blood through the ReliaPrep Blood gDNA Miniprep System (Promega), according to the manufacturer's recommendation. The Agilent SureSelect Custom Constitutional Panel 17 Mb (Agilent, Santa Clara, CA, USA) which provides comprehensive coverage of 5227 clinically relevant genes was used for the preparation of the library. Exon-enriched library was subjected to a 150 bp paired-end sequencing on the Illumina MiSeq platform (Illumina, San Diego, California, USA). Sequencing reads passing quality filters were aligned to the human reference genome build (GRGh37/hg19) and variant calling was performed using the SureCall v3.5 software (Agilent Technologies). Then VCF files were annotated with the wANNOVAR tool. Finally the variants were filtered and prioritized using a personalized bioinformatics pipeline: variants with a read coverage of less than 5x and a Qscore of below 20 were filtered out; allelic frequency in public databases variants <0.1% (1000 Genomes, the ESP cohort data set, GnomAD, Exome Aggregation Consortium, ExAC) were considered; synonymous variants were excluded; for the missense variants, we used at least four *in-silico* prediction tools (SIFT, CADD, Polyphen, MutationTaster).

2.10. Ethics and approval

The study was approved by the hospital's ethics committee (Milano Area 2, Prot. No. 829 2021 bis) and carried out in conformity with the 2013 revision of the Declaration of Helsinki. The subject gave her written consent to participate in the study.

3. Results and discussion

A 26-year-old woman who received the first dose of ChAdOx1 nCov-19 (AstraZeneca) vaccination 14 days earlier presented to the emergency room for headache, visual disturbance and difficulty walking. She rapidly developed right emiplegia. CT scan and MRI venography showed multifocal venous thrombosis with infarction and hemorrhagic transformation. The case report has been recently described in details by

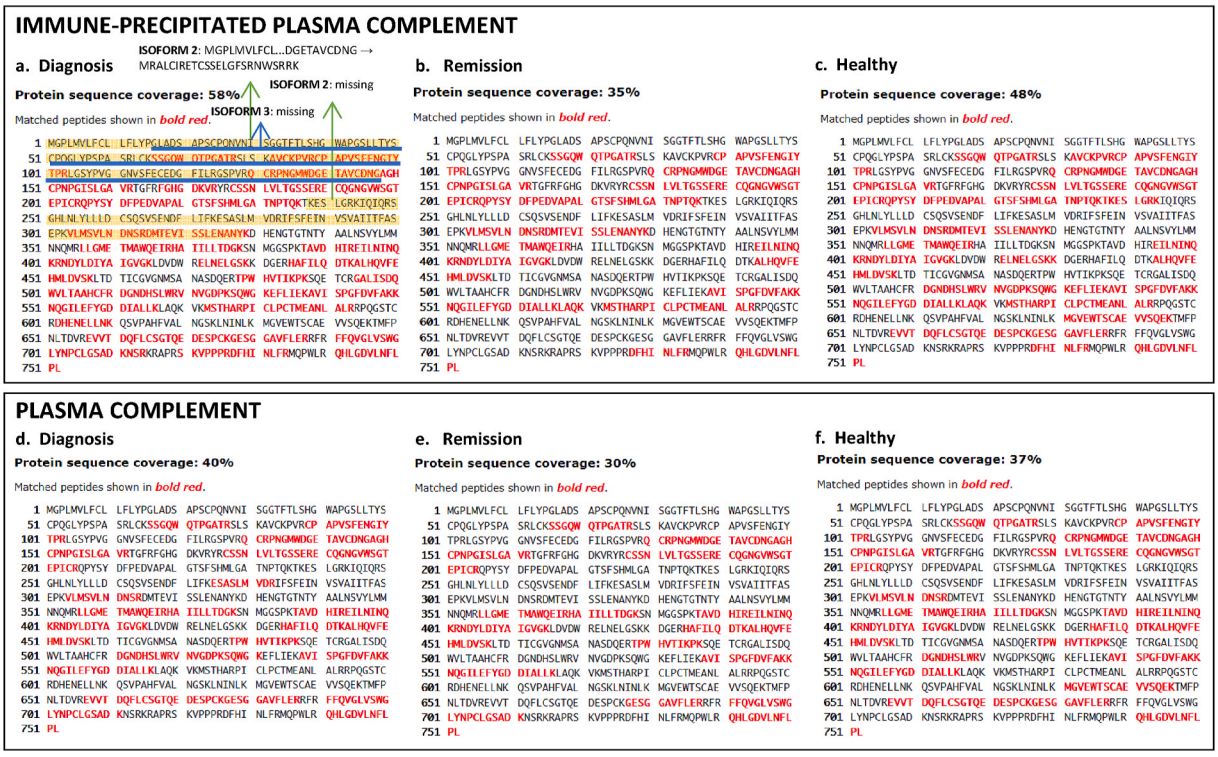


Fig. 2. Mass-spectrometry analysis of C2. Identified peptides (bold red) of immuno-precipitated (up) and circulating (down) complement C2 from plasma sample at the diagnosis (a and d), at the remission (b and e) and from a healthy subject (c and f). The sequences of isoform 2 and 3 are also highlighted in yellow and underlined in blue respectively (a).

Bonato et al. [7] according to CARE (CAse REport)-Statement and Checklist [8]. D-dimer was very high (12,204 μg/L; reference value < 500 μg/L), the platelet count was 134 × 10^9/L (reference values 150–450 × 10^9/L) and anti-PF4 IgG were present at high levels (1.918 OD; reference value < 0.400 OD). Given her recent exposure to ChAdOx1 nCoV-19 and clinical presentation, she was first treated with fondaparinux (5 mg subcutaneously) and admitted to ICU. IVIG ([Flebogamma DIF, Grifols, Barcelona, Spain] 1 g/kg o. d. for 2 days) and dexamethasone (40 mg/day, for 4 days) were started and fondaparinux was replaced by the short-acting drug argatroban [starting dosage 1 μg/kg x min with an aPTT-ratio (patient/normal) target of 1.5–2.0].

At admission, complement activities of the classical and mannose-binding lectin (MBL) pathways were absent (0% of normal with the Wieslab method) whereas complement activity of the alternative pathway was normal (73% of normal with the same method). The absence of activity of the classical complement pathway was confirmed by a hemolytic method (CH50 = 0). We observed normal levels of C3 (104% of normal), C1-inhibitor (activity 102% and antigen 130% of normal) and increased levels of C4 (195% of normal). C2 antigen levels were normal (59.8 μg/mL; reference values 50–100 μg/mL) but the immunoblotting analysis showed the disappearance of the normal band of C2 at about 110 kDa and a quite intense band at about 100 kDa (Fig. 1). The pattern of normal sera evidenced the presence of a marked band at 110 kDa but also a faint band at about 100 kDa, probably deriving from complement activation during serum production; for this reason, we hypothesized that the presence of the solely low molecular weight band in serum samples obtained from the patient at admission indicates the complete consumption of C2, explaining the absence of complement activities of both classical and MBL pathways. Moreover, proteomic analysis was performed aiming to investigate the presence of C2 isoforms in serum. The two C2 inactive isoforms are smaller than the typical/active C2 and they are both missing the amino acidic sequence responsible of the complement activity [9]. The results of the analyses performed on both whole serum and immune-precipitated C2 protein, showed the absence of peptides deriving from the two smaller isoforms of C2 (Fig. 2). As expected considering the immunoblotting pattern of C2, the mass-spectrometry data showed also the presence of both C2a and C2b fragments, not only in serum but also in purified samples, because anti-C2 antibody also immune-precipitates C2b and C2a in addition to residual C2 present in the samples. The possibility that the neutralization of the C2 activity was due to the presence of autoantibodies against C2 has been excluded on the basis of the absence of free anti-C2 antibodies and anti-C2 antibodies complexed to C2, evaluated by immunoenzymatic methods.

At admission, plasma levels of the complement terminal complex sC5b-9 were increased (630 ng/mL) compared to normal controls (139–462 ng/mL). Three days after IVIG infusion, we observed the normalization of the C2 pattern at immunoblotting and also normalization of the other complement parameters (Fig. 1). Moreover, plasma levels of anti-PF4 autoantibodies dropped by half (Fig. 1). The presence of C2 in the IVIG preparation was ruled out by an immunoenzymatic method. The absence of C2 in the immunoglobulin preparation and the normalization of sC5b-9, a reliable marker of *in vivo* complement activation, indicate that immunoglobulins *per se* have an effect on the mechanisms responsible for complement activation and consumption. The recovery of the classical and MBL pathways of complement after IVIG further supports this view. Seventeen days later, the beneficial effect of IVIG disappeared and we observed again the consumption of C2, the absence of activity of the classical and MBL pathways of complement as well as the increase of plasma levels of sC5b-9 and anti-PF4 autoantibodies (Fig. 1). The clinical and laboratory features normalized three days after the second infusion of IVIG and remained normal even 35 days later. In particular, a progressive decrease in plasma levels of anti-PF4 autoantibodies to values close to the upper limit of normal was observed at 35 days (Fig. 1).

In order to identify a genetic defect that predisposed the patient to this adverse reaction, we performed a Clinical Exome Sequencing analysis that in the first instance excluded the presence of pathological

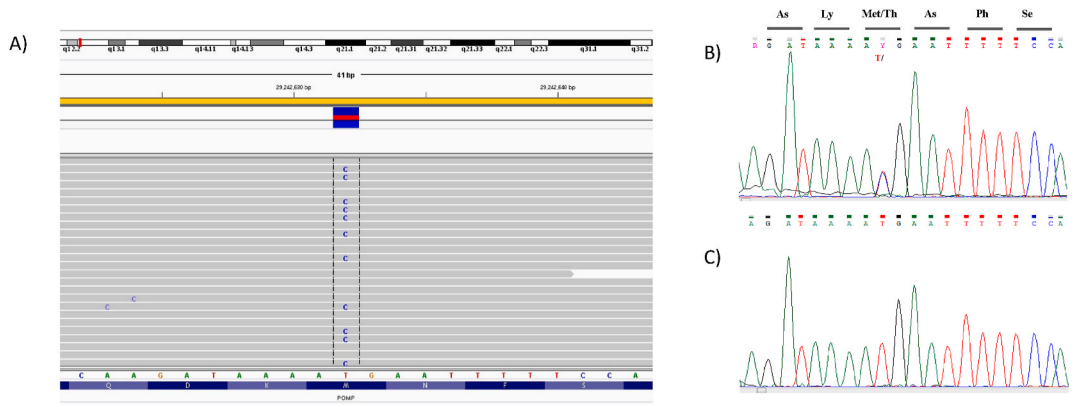


Fig. 3. Sequence analysis of POMP gene. Next Generation Sequencing file (A) and Sanger sequencing chromatograms (B,C) reporting the c. T185C variant in exon 4 (NM_015932.6) of *POMP* causing the p.Met62Thr substitution in the patient (B) and the corresponding wild-type sequence from a control sample (C).

alterations in the C2 gene and in the other complement proteins. Nevertheless, some variants with a possible clinical significance were identified (Supplementary Table S1) and among these, a nucleotide change in the Proteasome Maturation Protein *POMP* (c.T185C; Fig. 3) at the heterozygous state that determines the substitution of a methionine at position 62 with a Threonine (p.M62T). This variant is neither reported in the literature nor in the public databases (https://gnomad.broadinstitute.org/); it is predicted as deleterious by different in silico prediction tools (EIGEN, PROVEAN, MutationTesting) and classified VUS (Variant of Uncertain Significance) following the American College of Medical Genetics and Genomics guidelines (https://www.acmg.net). The parent's DNA was not available to establish the origin of this variant. *POMP* encodes for a chaperone protein involved in the formation of proteasome, the main cellular non-lysosomal proteolytic machinery that degrades polyubiquitinated proteins and maintains protein homeostasis. In particular POMP is essential for the formation of the 20S proteasome which is the proteolytically active component of the 26S proteasome complex. Upon the completion of proteasome maturation, POMP is degraded by the same proteasome. Monoallelic mutations in POMP resulting in proteasome deficiency cause Proteasome Associated Auto-inflammatory Syndromes (PRAAS) [10] an auto-inflammatory disorder characterized by chronic auto-inflammation neutrophilic dermatosis and fever. In addition Poli et al., in 2018 described a unique immune dysregulation syndrome in two unrelated individuals carrying *POMP* mutations presenting skin lesions, auotoantibodies, trombocytopenia, recurrent infections, high CD4/CD8 ratio and elevated immunoglobulin (IgG, IgA,IgE) levels [11]. It can be hypothesized that the here identified *POMP* variation might contribute along with other genetic and environmental factors to elicit a dysregulation to the proteasome function under a particular condition (as an immune response to the vaccine).

In conclusion, our results support the view that the absence of the activity of both classical and MBL pathways of the complement system and the C2 pattern observed at admission are not due to a genetic alteration of the C2 gene but rather to a strong complement activation that causes C2 consumption. Our data show complement activation in VITT and its normalization after IVIG treatment. Moreover, the relation between markers of complement activation and anti-PF4 antibody titer suggest the possible complement-activating capacity of anti-PF4 antibodies. In addition to the mechanism proposed by Mastellos et al. [3], in which C3 plays a pivotal role in the complement activation of VITT, we underly the involvement of C2 as limiting factor in complement activation observed in our patient. Our data support the potential contribution of complement activation to vaccine-related pathology and thus the need of monitoring complement parameters in these patients with the eventual possibility to target complement activation with specific drugs.

Author statement

Massimo Cugno designed the study and drafted the manuscript; Paolo Macor and Mara Giordano contributed to writing; Pier Luigi Meroni helped in the interpretation of data; Sara Bonato, Giacomo Comi, Andrea Artoni and Flora Peyvandi were responsible for the clinical management of the patient; Paolo Macor, Samantha Griffini, Elena Grovetti, and Luca De Maso performed complement studies, Mara Giordano and Simona Mellone performed genetic studies, Marcello Manfredi performed proteomics studies; Luca Valenti and Daniele Prati performed anti-PF4 studies. All authors contributed to the interpretation of the results, critically reviewed the manuscript, and approved the final version for submission.

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Author contributions

MC designed the study and drafted the manuscript; PM and MG contributed to writing; PLM helped in the interpretation of data; SB, GC, AA and FP were responsible for the clinical management of the patient; PM, SG, EG, and LDM performed complement studies, MG and SM performed genetic studies, MM performed proteomics studies; LV and DP performed anti-PF4 studies. All authors contributed to the interpretation of the results, critically reviewed the manuscript, and approved the final version for submission.

Declaration of competing interest

The authors have declared that no conflict of interest exists.

References

[1] A. Greinacher, T. Thiele, T.E. Warkentin, K. Weisser, P.A. Kyrle, S. Eichinger, Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination, N. Engl. J. Med. 384 (22) (2021) 2092–2101.
[2] D.B. Cines, J.B. Bussel, SARS-CoV-2 vaccine-induced immune thrombotic thrombocytopenia, N. Engl. J. Med. 384 (23) (2021) 2254–2256.
[3] D.C. Mastellos, P. Skendros, J.D. Lambris, Is complement the culprit behind COVID-19 vaccine-related adverse reactions? J. Clin. Invest. 131 (11) (2021), e151092.

[4] M. Cugno, R. Gualtierotti, I. Possenti, S. Testa, F. Tel, S. Griffini, E. Grovetti, S. Tedeschi, S. Salardi, D. Cresseri, P. Messa, G. Ardissino, Complement functional tests for monitoring eculizumab treatment in patients with atypical hemolytic uremic syndrome, J. Thromb. Haemostasis 12 (2014) 1440–1448, https://doi.org/10.1111/jth.12615.

[5] R.M. Dellepiane, L. Dell'Era, P. Pavesi, P. Macor, M. Giordano, L. De Maso, M. C. Pietrogrande, M. Cugno, Invasive meningococcal disease in three siblings with hereditary deficiency of the 8(th) component of complement: evidence for the importance of an early diagnosis, Orphanet J. Rare Dis. 11 (1) (2016) 64, https://doi.org/10.1186/s13023-016-0448-5.

[6] G. Dematteis, G. Vydmantaitė, F.A. Ruffinatti, M. Chahin, S. Farruggio, E. Barberis, E. Ferrari, E. Marengo, C. Distasi, R. Morkūnienė, A.A. Genazzani, M. Grilli, E. Grossini, M. Corazzari, M. Manfredi, D. Lim, A. Jekabsone, L. Tapella, Proteomic analysis links alterations of bioenergetics, mitochondria-ER interactions and proteostasis in hippocampal astrocytes from 3xTg-AD mice, Cell Death Dis. 11 (2020) 645, https://doi.org/10.1038/s41419-020-02911-1.

[7] S. Bonato, A. Artoni, A. Lecchi, G. Schwarz, S. La Marca, L. Padovan, M. Clerici, C. Guadino, G.P. Comi, A. Tripodi, F. Peyvandi, Massive cerebral venous thrombosis due to vaccine-induced immune thrombotic thrombocytopenia, Haematologica (2021), https://doi.org/10.3324/haematol.2021.279246 (Online ahead of print).

[8] D.S. Riley, M.S. Barber, G.S. Kienle, et al., CARE guidelines for case reports: explanation and elaboration document, J. Clin. Epidemiol. 89 (2017) 218–235.

[9] F.J. Milder, H. Raaijmakers, M. Vandeputte, A. Schouten, E. Huizinga, R. Romijn, W. Hemrika, A. Roos, M. Daha, P. Gros, Structure of complement component C2a: implications for convertase formation and substrate binding, Structure 14 (2006) 1587–1597, https://doi.org/10.1016/j.str.2006.08.008.

[10] F. Ebstein, M.C. Poli Harlowe, M. Studencka-Turski, E. Krüger, Contribution of the unfolded protein response (UPR) to the pathogenesis of proteasome-associated autoinflammatory syndromes (PRAAS), Front. Immunol. 10 (2019) 2756.

[11] M.C. Poli, F. Ebstein, S.K. Nicholas, M.M. de Guzman, L.R. Forbes, I.K. Chinn, E. M. Mace, T.P. Vogel, A.F. Carisey, F. Benavides, Z.H. Coban-Akdemir, R.A. Gibbs, S.N. Jhangiani, D.M. Muzny, C.M.B. Carvalho, D.A. Schady, M. Jain, J. A. Rosenfeld, L. Emrick, R.A. Lewis, B. Lee, , Undiagnosed Diseases Network members, B.A. Zieba, S. Küry, E. Krüger, J.R. Lupski, B.L. Bostwick, J.S. Orange, Heterozygous truncating variants in POMP escape nonsense-mediated decay and cause a unique immune dysregulatory syndrome, Am. J. Hum. Genet. 102 (6) (2018) 1126–1142.