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by Marco Capecchi, Pasqualina De Leo, Maria Abbattista, Ilaria Mancini, Pasquale Agosti, Marina Biganzoli, Chiara Suffritti, Barbara Ferrari, Anna Lecchi, Silvia La Marca, Lidia Padovan, Erica Scalambrino, Marigrazia Clerici, Armando Tripodi, Andrea Artoni, Roberta Gualtierotti, and Flora Peyvandi

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Risk of relapse after SARS-CoV-2 vaccine in the Milan cohort of thrombotic thrombocytopenic purpura patients

Running title: TTP relapse after SARS-CoV-2 vaccine

Marco Capecchi[1,2], Pasqualina De Leo[1], Maria Abbattista[1], Ilaria Mancini[3], Pasquale Agosti[3], Marina Biganzoli[1], Chiara Suffritti[1], Barbara Ferrari[1], Anna Lecchi[1], Silvia La Marca[1], Lidia Padovan[1], Erica Scalambrino[1], Marigrazia Clerici[1], Armando Tripodi[1], Andrea Artoni[1], Roberta Gualtierotti[1,3], Flora Peyvandi[1,3]

1 Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy

2 Clinica Moncucco, Division of Hematology, Lugano, Switzerland

3 Università degli Studi di Milano, Department of Pathophysiology and Transplantation and Fondazione Luigi Villa, Milan, Italy

Correspondence to:

Flora Peyvandi, MD, PhD, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico, Via Pace 9, 20122 Milan, Italy.

Phone +39 02-55035414

Fax +39 02-54100125

e-mail: flora.peyvandi@unimi.it

Authors contributions: M.C., R.G., A.T. and F.P. designed the study; M.C., A.A. and B.F. enrolled the patients; M.C. P.D.L. and M.A. performed the statistical analysis; M.C. wrote the manuscript;

all the other authors performed the laboratory tests. All authors critically revised and approved the last version of the manuscript.

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Dear Editor,

Thrombotic thrombocytopenic purpura (TTP) is a thrombotic microangiopathy characterized by reduced levels of ADAMTS13 (<10%) secondary to the presence of anti-ADAMTS13 autoantibodies in the acquired immune form (iTTP) or to ADAMTS13 gene mutations in the congenital form (cTTP). Acute episodes of TTP may be triggered by pregnancy, drugs such as oral contraceptives, and infections. TTP has been occasionally described also after vaccination. In the pre-COVID19 era, six cases of TTP have been reported after influenza, H1N1, pneumococcal and rabies vaccines, within two weeks. All cases but one were associated with vaccines against viral agents, and most of them (three) were associated with influenza vaccines, likely due to their wider availability (1). With the COVID-19 pandemic and the subsequent mass immunization program, safety concerns emerged about the possibility of TTP relapse after anti-SARS-CoV-2 vaccination. Since the availability of anti-SARS-CoV-2 vaccines, a total of 39 TTP cases (both first events or relapses) have been described, reporting a possible association between TTP onset and mRNA-based (Pfizer-BioNTech n=27, Moderna n=4), adenovirus vectors-based (AstraZeneca n=5, Janssen-Johnson & Johnson n=1) or inactivated whole-virus based (Sinopharm n=1, CoronaVac n=1) vaccines.

In this manuscript we report our single-center prospective cohort study aimed to evaluate the relapse rates in patients affected by TTP undergoing anti-SARS-CoV-2 vaccination. All consecutive adult TTP patients undergoing anti-SARS-CoV-2 vaccination from March to May 2021 were enrolled and observed until one month after the second dose. Multiple blood samples were collected: one week before the first dose of vaccination (T0), at least one week after the first and before the second dose (T1), and at least one week after and within a month from the second dose (T2). Patients were observed from T0 to T2 for clinical or ADAMTS13 relapse (decrease in activity to <20%). Venous blood samples were tested for whole blood count, ADAMTS13 activity

(2), anti-ADAMTS13 antibodies, prothrombotic markers (FVIII:C, VWF:Ag and D-dimer plasma levels), anticoagulant markers (protein C activity), anti-PF4 and anti-S antibodies.

Data on demographics, type of vaccine and immunosuppression treatment were collected. Categorical variables were expressed as counts and percentages and continuous variables as mean and standard deviation or median and interquartile range (IQR). Continuous variables at the different timepoints were compared by repeated measures ANOVA for normally distributed and Kruskal-Wallis test for non-normally distributed variables.

A total of 49 TTP patients were enrolled, 37 females and 12 males, in line with the reported 3:1 female prevalence of the disease with a median age of 50 years (IQR 40-59 years). All patients were vaccinated with the Pfizer-BioNTech mRNA BNT162b2-Comirnaty vaccine. Forty-eight patients were affected by iTTP, while one had cTTP. The latter did not develop any clinical relapse and did not show any variation of the ADAMTS13 levels at the different timepoints. At baseline all iTTP patients were in clinical remission and the median plasma levels of ADAMTS13 were 62% (IQR 34-87%). At T0 ADAMTS13 activity <20% was observed in 5 (10%) patients, 2 of which with activity <10%, while 9 (19%) patients had activity between 20 and 45%. Among patients with ADAMTS13 plasma levels below the lower limit of the normal range, only one had borderline anti-ADAMTS13 antibodies (15IU/mL; normal range <12, borderline 12-15IU/mL).

Within one month from the second vaccine dose, no patients had a clinical TTP relapse and only one had an ADAMTS13 relapse with plasma levels <10%. Mean levels of ADAMTS13 activity were stable among the 3 timepoints (**Figure**). In only two patients a significant decrease of ADAMTS13 levels occurred after the first dose (from 28% to <3% and from 101% to 82%), and both remained stable after the second dose, with negative anti-ADAMTS13 antibodies. Notably, even though with undetectable ADAMTS13 plasma levels, the first patient received also the second vaccine dose, that didn't elicit a clinical relapse. Due to a stable undetectable ADAMTS13 he was treated with

375mg/m2 rituximab once weekly for 4 weeks showing a rapid ADAMTS13 response. Rituximab was started 1 month after the second dose to maximize the serological response to vaccination. One patient had positive basal anti-ADAMTS13 antibodies with a titer remaining stable after the two vaccine doses, while in another patient anti-ADAMTS13 antibodies became detectable after the first dose, with no corresponding drop in ADAMTS13 levels and a stable titer after the second dose.

Among patients with iTTP, 21 were treated with an immunosuppressive drug over the last year before enrollment. Of those, most patients were treated with only one immunosuppressive drug, while 4 patients with 2, and 3 patients with 3 different drugs. Thirteen patients had been treated with 4 to 6 infusions of rituximab with the standard schedule of 375mg/mq weekly, 8 patients with steroids (prednisone or metilprednisolone) at variable doses (from 1mg/kg to low maintenance doses: 5mg once-daily), 3 with hydroxychloroquine at the standard dose of 200mg once-daily, 6 with azathioprine at a dose of 1.5-2.0mg/kg once-daily, 2 with cyclosporine at a dose of 1.5-2mg/kg/day. Nine out of 13 patients had received the last dose of rituximab within 9 months of the first vaccine dose (3). Eleven patients were on immunosuppressive treatments at the time of the first vaccine dose. None of the patients at the time of the first vaccine dose were on more than 10mg of prednisone equivalent dose, as previously recommended (4, 5).

Anti-PF4 antibodies were negative in all patients except one at T2. This patient was not exposed to heparin and did not show any other sign or symptom suggestive of vaccine-induced thrombotic thrombocytopenia (VITT). Indeed, no confirmed cases of VITT associated with mRNA vaccines have been reported in the literature (6).

Six patients showed a positive titer of anti-spike antibodies before the first dose of vaccine. No systematically collected data on previous exposure to SARS-CoV-2 are available. After the first vaccine dose, 33 patients became positive, and 9 more patients became positive after the second

dose. A total of 5 (10%) patients did not show a serological response to the two doses of vaccine. Of those, 2 patients had received the last dose of rituximab within nine months from the first vaccine dose (2 and 4 months) and one patient was on continuous treatment with cyclosporine. For one patient who resulted negative after the first dose any serum sample was available after the second dose to evaluate the antibody response. A statistical analysis conducted with Student's t test showed no significant difference between the patients that received immunosuppressive treatment in the year before the first vaccine dose and those off treatment in the levels of anti-spike antibodies titers.

Concerning the procoagulant parameters FVIII:C, VWF:Ag and D-dimer, no statistically significant differences were found in plasma levels at the three timepoints. No difference was found for the natural anticoagulant protein C plasma levels, as well. No significant changes in white blood cells or platelet count at the three timepoints were observed (**Table 1**).

Due to the inflammatory response induced by vaccines, a possible role of vaccines in induction of autoimmune diseases has been proposed, via different mechanisms such as molecular mimicry and polyclonal immune response (7). So that vaccines may represent a trigger for TTP as well, being an autoimmune disease, even though only sporadic cases of acute TTP after vaccination have been reported in the literature so far. In our cohort any patient developed a clinical relapse and only one out of 48 developed an ADAMTS13 relapse in the observation period for a rate of 1.36% per month, compared with the 2.6% clinical relapse rate reported in the literature (8) and a 0.52 incidence rate observed/expected. Our results are in line with the results of 2 multicenter studies that showed an incidence rate of TTP relapse or new onset within 4 weeks after vaccination lower than expected in the vaccinated population (9, 10). Conversely, we observed fewer relapses than in another Italian monocentric study (overall clinical/ADAMTS13 relapse 2% vs 13% of cases) (11). Of note, although the proportion of patients with baseline ADAMTS13 activity

below normal was similar (29% vs 31%), those of patients with baseline ADAMTS13 activity <20%, who are supposed to be at higher risk of TTP relapse after a trigger, was significantly lower in our study (10% vs 22%), possibly explaining the observed differences.

Overall, the analysis of the coagulation activation showed no increase of the procoagulant factors such as FVIII:C and VWF:Ag, suggesting that anti-SARS-CoV-2 vaccines do not induce an inflammatory response strong enough to determine a hypercoagulable state, in contrast to what is induced by the virus itself (12, 13).

In conclusion, the results of our study prospectively evaluating the effect of anti-SARS-CoV-2 vaccination on the risk of relapse in a large cohort of patients with TTP in Milan showed a lower than reported relapse rate (1.36% vs 2.6%) with an observed/expected incidence rate ratio of 0.52, confirming the safety of mRNA-based anti-SARS-CoV-2 vaccination in TTP patients. Moreover, while the association of TTP relapse with any kind of mRNA vaccination is negligible, the association with infection, especially if characterized by a strong inflammatory response, is much higher (31% of TTP relapses in our historical cohort) (14). Indeed, many reports on COVID-19-associated TTP have been reported since the pandemic onset.

Based on our results, patients with TTP may receive safely the anti-SARS-CoV-2 vaccination. However, due to the reported cases of TTP relapse after vaccination, it is of pivotal importance to carefully evaluate the platelet count and ADAMTS13 levels before and after the vaccination, with a more strictly monitoring for patients with lower levels at baseline.

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Age (years), median (IQR)	50 (40-59)
Sex, n (%)	
Male	12 (25)
Female	37 (77)
Number of TTP episodes, median (min-max)	1 (1-7)
Time from last TTP episode to first vaccine dose (years), median (IQR)	5 (3-9)
Immunosuppression therapy in the year before vaccination, n (%) *	21 (43)
Rituximab, n (%)	13 (27)
Steroids, n (%)	8 (17)
Azathioprine, n (%)	7 (15)
Cyclosporine, n (%)	1 (2)
Hydroxycloroquine, n (%)	3 (6)
Time from last rituximab to 1° vaccine dose (months), median (IQR)	6 (4-12)
Ongoing immunosuppression at 1° dose, n (%)	11 (23)

Table 1. Demographic and clinical characteristic of aTTP patients. IQR, interquartile range; TTP, thrombotic thrombocytopenic purpura; n, number.

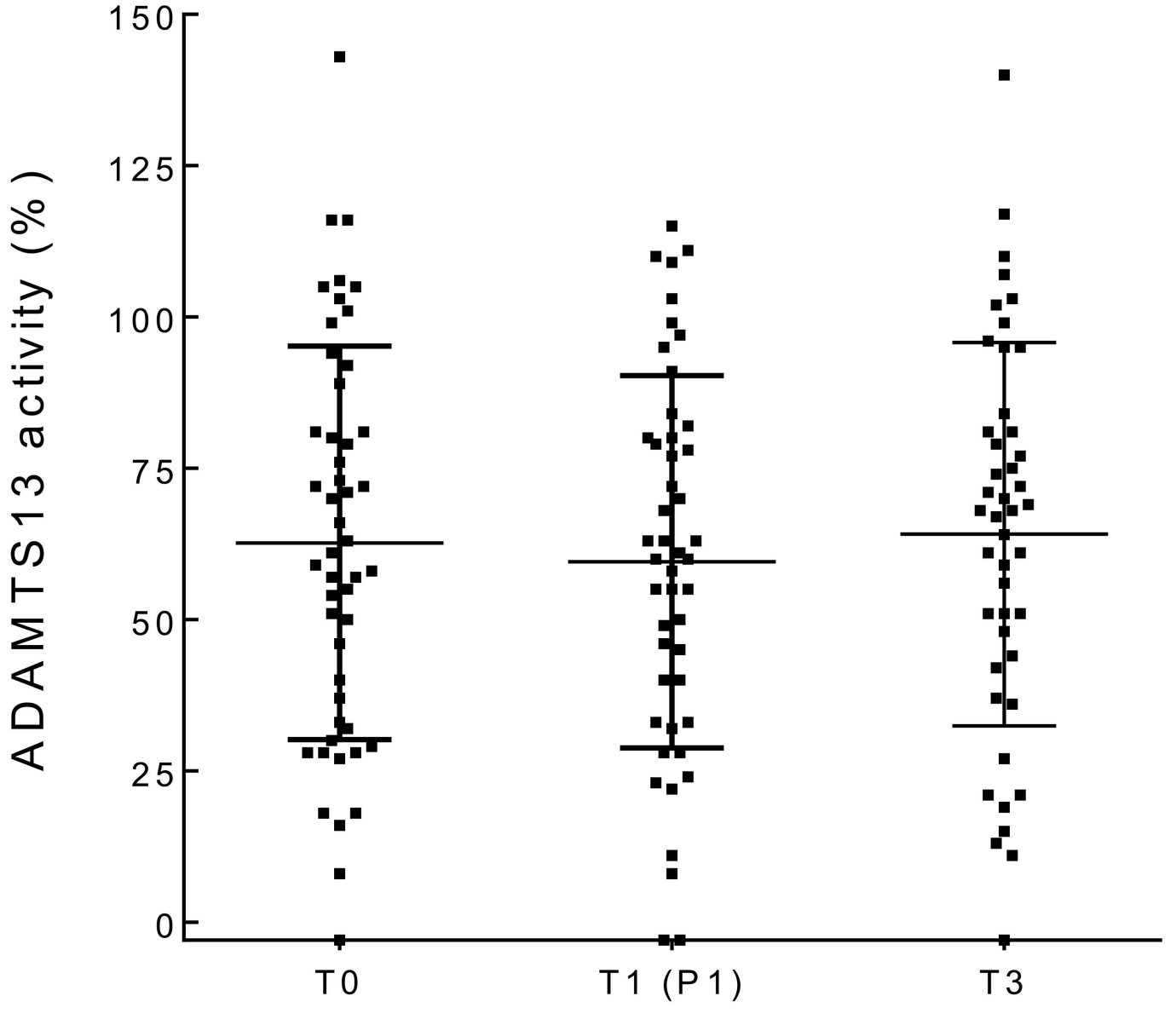
* some patients were on concomitant treatment with more than one immunosuppressive agent.

	T0	T1	T2
ADAMTS13 (%), median (IQR)	61 (33-81)	60 (37-80)	68 (43-81)
Anti-ADAMTS13 antibodies, n (%)	1 (2%)	2 (4%)	2 (4%)
Anti-PF4 antibodies, n (%)	0	0	1 (2%)
Anti-Spike antibodies, n (%)	6 (13%)	33 (69%)	35 (73%)
Platelet count ($x10^3$/uL), median (IQR)	240 (216-293)	259 (226-310)	260 (225-304)
FVIII:C (%), median (IQR)	85 (66-105)	82 (67-100)	84 (73-104)
VWF:Ag (%), median (IQR)	110 (90-138)	113 (85-132)	117 (91-138)
D-dimer (FEU), median (IQR)	290 (157-387)	246 (182-401)	249 (169-376)
Protein C (%), median (IQR)	96 (84-109)	92 (82-108)	95 (82-115)

Table 2. Laboratory characteristics of aTTP patients. No statistically significant differences were observed between the three timepoints for ADAMTS13 and hemostatic parameters median levels. IQR, interquartile range; n, number; FVIII:C, factor VIII coagulant activity; VWF:Ag, von Willebrand factor antigen.

Figures Legend:

Figure. Plasma levels of ADAMTS13 in TTP patients before (T0), two weeks after the first dose (T1) and two weeks after the second dose (T2) of anti-SARS-CoV-2 vaccination. Horizontal bars represent mean and standard deviation. ns, non-statistically significant.


ADAMTS13 activity (%)
150
125
100
75
50
25
0
T0
T1 (P1)
T3