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

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 were reported after influenza, H1N1, pneumococcal and rabies vaccines, within 2 weeks. All cases but one were associated with vaccines against viral agents, and most of them (3) 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 1 month after the second dose. Multiple blood samples were collected: 1 week before the first dose of vaccination (T0), at least 1 week after the first and before the second dose (T1), and at least 1 week after and within 1 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 (Table 1). 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 time points 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 time points. 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 five (10%) patients, two of which with an activity <10%, while nine (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 (15 IU/mL; normal range <12 IU/mL, borderline 12-15 IU/mL).

Within 1 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 three time points (Figure 1). 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 sec-

Table 1. Demographic and clinical characteristics of acute thrombotic thrombocytopenic purpura patients.

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

*Some patients were on concomitant treatment with more than one immunosuppressive agent. IQR: interquartile range; TTP: thrombotic thrombocytopenic purpura; N: number.

ond 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 375 mg/m^2 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 four patients with two, and three patients with three different drugs. Thirteen patients had been treated with four to six infusions of rituximab with the standard schedule of 375 mg/mq weekly, eight patients with steroids (prednisone or metilprednisolone) at variable doses (from 1 mg/kg to low maintenance doses: 5 mg once-daily), three with hydroxychloroquine at the standard dose of 200 mg once-daily, six with azathioprine at a dose of 1.5-2.0 mg/kg once-daily, two with cyclosporine at a dose of 1.5-2 mg/kg/day. Nine 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 10 mg 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 nine more patients became positive after the second dose. A total of five (10%) patients did not show a serological response to the two doses of vaccine. Of those, two patients who had received the last dose of rituximab within 9 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 no 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.

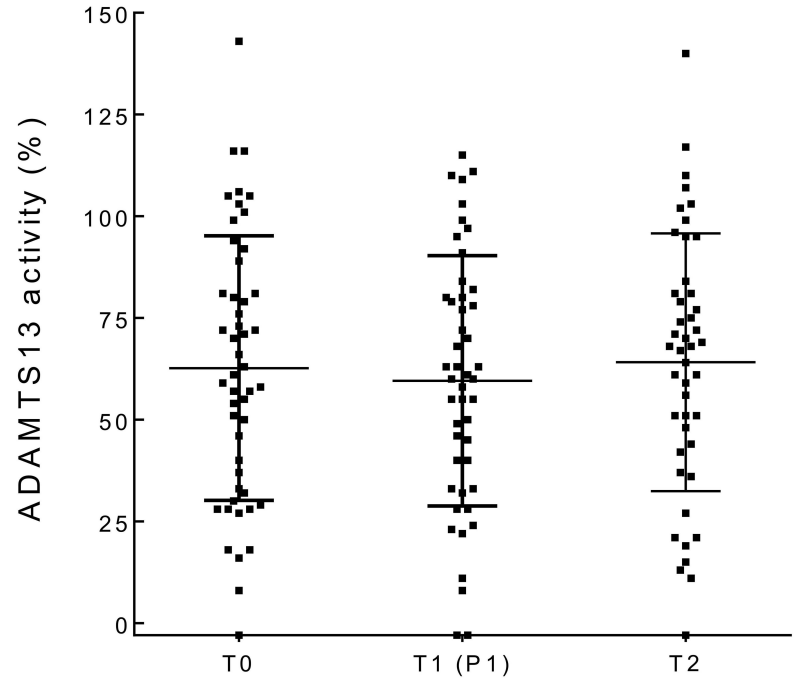

Figure 1. Plasma levels of ADAMTS13 in thrombotic thrombocytopenic purpura patients before (T0), 2 weeks after the first dose (T1) and 2 weeks after the second dose (T2) of anti-SARS-CoV-2 vaccination. Horizontal bars represent mean and standard deviation.

Concerning the procoagulant parameters FVIII:C, VWF:Ag and D-dimer, no statistically significant differences were found in plasma levels at the three time points. 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 time points were observed (Table 2).

Due to the inflammatory response induced by vaccines, a possible role of vaccines in the induction of autoimmune diseases has been proposed, via different mechanisms such as molecular mimicry and polyclonal immune response.[7] Therefore 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 no patient developed a clinical relapse and only one 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 two multi-center 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 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%), the proportion of patients with a baseline ADAMTS13 ac-

Table 2. Laboratory characteristics of acute thrombotic thrombocytopenic purpura patients.

Characteristics	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 x10^9/L, 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)

No statistically significant differences were observed between the 3 time points for ADAMTS13 and hemostatic parameters median levels. TO: before the first vaccination; T1: 2 weeks after the first dose of vaccination; T2: 2 weeks after the second dose; IQR: interquartile range; N: number; FVIII:C: factor VIII coagulant activity; VWF:Ag: von Willebrand factor antigen; FEU: fibrinogen equivalent units.

tivity of <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 safely receive 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 more strictly monitoring for patients with lower levels at baseline.

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Disclosures

IM received honoraria for participating as a speaker at educational meetings organized by Instrumentation Laboratory and Sanofi. AA received honoraria for participating as speakers at educational meetings organized by Sanofi. FP has received honoraria for participating as a speaker in education meetings organized by Grifols and Roche, and she is member of scientific advisory boards of Sanofi, Sobi, Takeda, Roche, Biomarin. The other authors do not have any conflicts of interests to disclose.

Contributions

MC, RG, AT and FP designed the study. MC, AA and BF enrolled the patients. MC, PDL and MA performed the statistical analysis. MC wrote the manuscript; all the other authors performed the laboratory tests. All authors critically revised and approved the last version of the manuscript.

Data-sharing statement

Original data will be made available upon request.

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