blood advances

TO THE EDITOR:

Efficacy and safety of azathioprine during remission of immune-mediated thrombotic thrombocytopenic purpura

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Acquired immune-mediated thrombotic thrombocytopenic purpura (iTTP) is a rare and potentially fatal thrombotic microangiopathy caused by the development of anti-ADAMTS13 autoantibodies.[1-8] Up to 50% of patients surviving an acute iTTP event will experience a clinical relapse with an associated mortality risk.[9-11] Patients with low ADAMTS13 activity during remission are at high risk of relapse and should be promptly treated with an immunosuppressive regimen, rituximab being the first-choice drug.[12-14] It has been reported that 10% of cases that do not respond to rituximab or develop allergic reactions require therapy discontinuation.[15,16] In them, it is not clear which drug is more appropriate as a second-line treatment. Azathioprine blocks purine production, preventing replication of highly proliferating cells such as B and T lymphocytes.[17,18] It has been used to treat many autoimmune diseases, but scant evidence exists on its use for patients with iTTP.[19] With this background and gap of knowledge, we assessed azathioprine efficacy and safety for relapse prevention in a cohort of patients with iTTP in clinical remission.

We designed a single-arm cohort study in which patients enrolled in the Milan TTP registry between January 2002 and October 2020 were retrospectively screened for the following inclusion criteria: (1) iTTP diagnosis confirmed by evidence of severe ADAMTS13 deficiency (ADAMTS13 activity $<10\%$) and anti-ADAMTS13 antibodies, or ADAMTS13 activity normalization after the acute event; and (2) treatment with azathioprine during iTTP clinical remission. Patients with incomplete data related to azathioprine exposure were excluded. Patients were followed until the end of azathioprine treatment or loss to follow-up or the end date of our study (set at 30 April 2021), whichever occurred first.

Azathioprine efficacy during iTTP remission was assessed in patients treated for $\geq$1 month, this being the time required for azathioprine to be effective.[20] Our primary efficacy outcome was cumulative incidence of clinical relapse (defined according to Scully and colleagues[21]) occurring during azathioprine treatment. Secondary efficacy outcomes were partial and complete ADAMTS13 remission (ADAMTS13 activity increase above or equal to 20% and 45%, respectively) and ADAMTS13 relapse (ADAMTS13 activity decrease below 20%), according to Cuker and colleagues.[22] Only patients with ADAMTS13 activity results at baseline (ie, before azathioprine start or within 1 month since azathioprine start) and $\geq$4 weeks after starting azathioprine were included. To assess azathioprine safety, we performed a chart review recording all adverse events (AEs) occurring during azathioprine treatment. Written informed consent was obtained from all subjects with the approval of the Ethics Committee of our institution in accordance with the Declaration of Helsinki.

We identified 48 iTTP patients treated with azathioprine during remission between 2002 and 2020 (supplemental Figure 1). We excluded 8 of them for insufficient clinical data regarding azathioprine exposure or lack of evidence of severe ADAMTS13 deficiency. Baseline characteristics of the 40 included patients

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For original, deidentified data, please contact the corresponding author.

The full-text version of this article contains a data supplement.

Table 1. Patient characteristics

Demographics and baseline characteristics	n = 40
Age at first iTTP episode (yr), median (IQR)	47 (32-54)
Age at azathioprine start (yr), median (IQR)	49 (38-58)
Female sex, n (%)	31 (78)
Ethnicity, n (%)	
Caucasian	39 (98)
Black	1 (2)
0 blood group, n (%)*	13 (46)
BMI (kg/m^2), median (IQR)	26 (23-31)
First iTTP episode, n (%)	15 (38)
ADAMTS13 activity before azathioprine start, n (%)†	
ADAMTS13 <10%	20 (62)
10%≤ADAMTS13<20%	5 (16)
20%≤ADAMTS13<45%	5 (16)
ADAMTS13 ≥45%	2 (6)
Exposure, median (IQR)	
Azathioprine dosage, mg/kg per d	1.3 (1-1.6)
Duration of azathioprine treatment, mo	16 (8-45)
Autoimmune comorbidities, n (%)	
Patients with ≥1 autoimmune comorbidity	15 (38)
Patients with:	
1 autoimmune comorbidity	12 (30)
2 autoimmune comorbidities	2 (5)
3 autoimmune comorbidities	1 (2)
List of autoimmune comorbidities, n (%)	
Hashimoto thyroiditis	9 (47)
Systemic lupus erythematosus	3 (16)
Raynaud disease	2 (11)
Evans syndrome	1 (5)
Chronic urticaria	1 (5)
Graves' disease	1 (5)
Sjogren's syndrome	1 (5)
Immune thrombocytopenic purpura	1 (5)

BMI, body mass index.
*Available for 28 patients.
†Available for 32 patients.

are shown in Table 1. The majority (60%) was treated during the remission of a relapsing episode. Baseline ADAMTS13 activity was available in 32 patients, 25 of whom had activity levels <20%. Reasons why azathioprine was given to patients with mildly reduced to normal ADAMTS13 activity or regardless of the availability of ADAMTS13 testing results are detailed in supplemental Table 1.

Twenty-five patients were given azathioprine after rituximab failure and the median time from the last infusion to azathioprine start was 13 months. Rituximab was usually given as a 375 mg/m^2 4-infusion cycle. Twelve patients were given azathioprine instead of or before rituximab to prevent iTTP relapse because rituximab was not yet part of clinical practice at the time, and its use in Italy is authorized only for refractory and relapsing iTTP. Three patients were given azathioprine to treat another autoimmune comorbidity: 1 was diagnosed with iTTP and Sjogren's syndrome at the time of the acute iTTP event, and the remaining 2 were diagnosed with systemic lupus erythematosus before iTTP development and had already been on azathioprine for 6 and 21 years.

Thirty-five patients were included in our primary efficacy outcome analysis, with a median follow-up time of 40 months (95% confidence interval [CI], 14-69); details for exclusion are given in supplemental Figure 1. Clinical relapse occurred in 20% of the patients. Cumulative incidence of clinical relapse calculated with the Kaplan-Meier method was 10% at 1 year (95% CI, 3-27%) and 22% at 2 years (95% CI, 10-43%). The same analysis performed on the subgroup of patients with baseline ADAMTS13 activity <20% (thus at higher relapse risk) yielded similar results: 19% relapsed, with a 1- and 2-year cumulative incidence of relapse of 6% (95% CI, 0-18) and 21% (95% CI, 0-42), respectively. These results suggest that azathioprine is effective in reducing iTTP relapses compared with the expected 30% to 50% relapse rate.[9,11]

Among the 32 patients with available baseline ADAMTS13 activity, 25 with regular ADAMTS13 samples and a more-than-1-month exposure to azathioprine were included in our secondary efficacy outcomes analysis (Table 2 and supplemental Figure 2). Among the

Table 2. Secondary efficacy outcomes and AEs

Secondary Efficacy Outcomes	n = 25
Baseline ADAMTS13 <20%	n = 21
Partial remission, n (%)	10 (48)
Time to partial remission (mo), median (IQR)	3.0 (2.8-8.5)
Duration of partial remission (mo), median (IQR)	40 (16-56)
Complete remission, n (%)	7 (33)
Time to complete remission (mo), median (IQR)	8 (3-16)
Duration of complete remission (mo), median (IQR)	16 (0-53)
Baseline ADAMTS13 ≥20%	n = 4
ADAMTS13 relapse, n (%)	3 (75)
AEs, n (%)	n = 40
Patients with ≥1 AE	11 (28)
Patients with 1 AE	6 (15)
Patients with 2 AEs	5 (13)
AEs leading to drug discontinuation	5 (13)
List of AEs, n (%)	
Gastrointestinal	8 (44)
Hepatopancreatic	5 (28)
Leukopenia	2 (11)
Acute myeloid leukemia	1 (6)
Joint pain	1 (6)
Oral aphthosis	1 (6)
List of AEs leading to azathioprine stop, n (%)	
Hepatopancreatic	4 (50)
Gastrointestinal	3 (38)
Acute myeloid leukemia	1 (13)
Time to adverse events (d), median (IQR)*	50 (27-332)

*Available for 9 patients.

21 patients with baseline ADAMTS13 activity <20%, 10 (48%) attained a partial ADAMTS13 remission after a median time of 3 months (interquartile range [IQR], 2.8-8.5 months) and with a median response duration of 40 months (IQR, 16-56 months). Complete remission occurred in 33% of the 21 patients. Among the 10 patients who attained ADAMTS13 remission, 3 had a subsequent ADAMTS13 relapse after 22, 24, and 37 months but without a clinical relapse. Two of them were maintained on azathioprine, and 1 switched to mycophenolate. In the second group of 4 patients with baseline ADAMTS13 activity ≥20%, 3 had an ADAMTS13 relapse after 2, 3, and 13 months. Autoantibodies were monitored during treatment, with results usually paralleling ADAMTS13 activity trends.

Most patients with low baseline ADAMTS13 activity attained a durable response (40 months). A plausible explanation is that daily azathioprine assumption maintains the underlying autoimmune process under control, whereas rituximab is usually given as a 4-week cycle, with an expected median remission of 18 months, so that its efficacy wanes over time, potentially requiring retreatment.[15]

All 40 patients were included in the safety analysis: 28% had ≥1 AE and 13% discontinued azathioprine. The most frequent AEs were gastrointestinal and hepatopancreatic toxicities. Two patients developed leukopenia, but neither anemia nor an increased susceptibility to infections was recorded, probably because of the moderate dosage employed. A patient developed acute myeloid leukemia 5 months after starting azathioprine: this could be because of azathioprine itself or as an independent process.[23]

Our study has limitations. It is a retrospective cohort study with an enrollment period spanning a long period of time. The sample size was limited because of disease rarity and the varied frequency of ADAMTS13 testing during remission. We might have underestimated the prevalence of AEs since they were captured retrospectively neither in a standardized way nor at standard time points. Finally, the unique characteristics of our cohort (eg, the high prevalence of autoimmune comorbidities) might limit the generalizability of our findings to other iTTP populations.[24]

In conclusion, azathioprine should be considered the second-line treatment for iTTP relapse prevention in patients unresponsive or intolerant to rituximab since a durable ADAMTS13 remission was achieved in half of the patients and with infrequent and relatively mild AEs. Confirmation of our results from larger samples and randomized controlled trials is warranted to compare different immunosuppressive drugs for iTTP relapse prevention for patients in remission who are unresponsive to rituximab.

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