Thrombophilia screening in patients with autoimmune hemolytic anemia: a single-center analysis

Thrombotic complications have been reported in approximately 15–20% of patients with autoimmune hemolytic anemia (AIHA).[1-6] Contributing factors likely include nitric oxide scavenging by circulating free hemoglobin, the release of erythrocyte-derived microparticles,[7-9] and a multifaceted interplay among autoimmunity, autoinflammation, complement activation, and the coagulation system, as well as coexisting conditions. A recent systematic review estimated a pooled risk ratio of 2.63 (95% confidence interval: 1.37–5.05) for thrombotic events in AIHA.[7] Retrospective series have identified several clinical risk factors for thrombosis, including severe anemia at onset (hemoglobin <6 g/dL), intravascular hemolysis (lactate dehydrogenase >1.5× the upper limit of normal), prior splenectomy, and the presence of Evans syndrome.[5,10-13] However, the role of routine thrombophilia screening in AIHA has not been established yet. The primary objective of this study was to assess the prevalence of inherited and acquired thrombophilia in AIHA and its association with incident thrombotic events. Secondary objectives included estimating incidence rates of thrombosis and evaluating the impact of antithrombotic prophylaxis.

We systematically enrolled a cohort of 92 patients with confirmed AIHA according to current guidelines.[1] Patients underwent a thorough thrombophilia screening including the collection of an accurate personal and family history of thrombosis (venous thrombotic events [VTE]/arterial thrombotic events [ATE]) and of anamnestic risk factors, followed by sampling of peripheral blood for inherited and acquired forms. Thrombotic complications were classified as occurring prior to or after the onset of the AIHA and as venous or arterial. Additionally, anticoagulant or antiplatelet prophylaxis during thrombotic episodes was recorded, allowing evaluation of the incidence of thrombosis according to the ongoing prophylaxis. The study was approved by the ethical committee as a substudy of the CYTOPAN trial (NCT05931718).

The median age at the first AIHA episode was 60 years (range, 45-72) and 59% of the patients were females (Table 1). Most patients had warm AIHA (58%), with a direct antiglobulin test positive for IgG+C in one-third, followed by cold agglutinin disease (31%), and the rarer mixed and atypical forms (11%). During the retrospective follow-up, the patients experienced a median of two (range, 1-4) hemolytic episodes.

Thrombophilia abnormalities were detected in 20/92 patients (22%), including deficiencies of antithrombin, protein C, or protein S, observed in 6/92 (7%); factor V Leiden or prothrombin G20210A mutations in one patient each; and antiphospholipid antibodies in 10/92 (11%). Of these ten with antiphospholipid antibodies, five were triple-positive, two were double-positive, two had lupus anticoagulant only, and one had isolated anticardiolipin positivity. Other acquired risk factors for thrombophilia that were evaluated

Table 1. Baseline characteristics of the study population at the first episode of autoimmune hemolytic anemia.

Characteristics	All patients N=92
Male/female, N (%)	38 (41)/54 (59)
Age at first AIHA episode, years, median (IQR)	60 (45-72)
ABO group, O /non-O, N	34/37
N of hemolytic events, median (IQR)	2 (1-4)
AIHA thermal classification, N (%)	
Warm	53 (58)
IgG	37 (69)
IgG+C	16 (31)
Cold	29 (31)
Mixed/atypical	10 (11)
AIHA type, N (%)	
Idiopathic	65 (71)
Autoimmunity	17 (18)
Lymphoproliferative disorder	7 (8)
Other	1 LR-MDS, 1 PNH, 1 MPN
Evans syndrome	8* (9)
Lines of treatment, median (range)	2 (1-6)
Type of treatment, N (%)	
Steroids	89 (97)
Rituximab	72 (78)
Splenectomy	0 (0)
Cytotoxic immunosuppressants	5 (5)
Others	14** (15)

*Seven warm, one mixed/atypical. **Nipocalimab, isatuximab, obexelimab, sutimlimab, parsaclisib and fostamatinib. Autoimmune hemolytic anemia (AIHA) was classified according to positivity of the direct antiglobulin test (DAT) into warm (IgG or IgG+C3d), cold (C3d and agglutinin titer >64), mixed (IgG+C3d and cold agglutinin titer >64), and atypical cases (IgA-positive or DAT-negative ones). AIHA was idiopathic in 71% of cases, and secondary to lymphoproliferative disorders, autoimmune diseases, myelodysplastic syndrome, paroxysmal nocturnal hemoglobinuria or myeloproliferative neoplasm in the remaining; eight patients had Evans syndrome. Patients received a median of two lines of treatment (range, 1-6), with corticosteroids (N=89) and rituximab (N=72) being the most commonly administered therapies. No patients underwent splenectomy, while cytotoxic immunosuppressants (N=5) and other treatments (N=14) were used less frequently. IQR: interquartile range; C: complement; LR-MDS: low-risk myelodysplastic syndrome; PNH: paroxysmal nocturnal hemoglobinuria; MPN: myeloproliferative neoplasm.

included combined oral contraceptive use, reported in 26/54 women (48%), previous cancer in 14 (15%), recent surgery or trauma in 50 (54%), cardiovascular disease risk factors in 40 (43%), and autoimmune comorbidities in 21 (23%). A personal history of VTE/ATE was documented in ten patients (11%), while a positive family history was noted in 50 cases (54.3%). Concerning conditions associated with AIHA that may increase the thrombotic risk, eight patients had Evans syndrome, one patient had a classic hemolytic paroxysmal nocturnal hemoglobinuria with high disease activity (lactate dehydrogenase 1.5 x upper limit of normal and anemia), and one had essential thrombocythemia with a platelet count of $900x10^9$/L while on treatment with hydroxyurea. Finally, a total of three pregnancies occurred after the diagnosis of AIHA among 54 women: one was in a woman who had a prior pregnancy with intrauterine growth restriction, who delivered successfully without complications; a second woman, with an uncomplicated pregnancy history before AIHA, experienced intrauterine growth restriction during her post-AIHA pregnancy but then completed it successfully; the third woman had a hydatidiform mole after the AIHA diagnosis and interrupted her pregnancy. Notably, thrombophilia screening was performed at a median of 4 years (IQR, 0-39 years) after the diagnosis of AIHA, thus limiting the reliability of the acquired risk factors in this analysis.

Among the 92 patients, 20 (22%) experienced a total of 29 thrombotic events, of which 11 (8 VTE and 3 ATE) occurred at diagnosis of the AIHA. Among those with VTE at AIHA onset, three patients had concurrent Evans syndrome, and two had a prior history of VTE. An additional 18 thrombotic events occurred after the AIHA diagnosis, consisting of 14 VTE and four ATE. Of the 14 VTE, 12 occurred during AIHA relapses while two occurred during remission from the AIHA. Table 2 compares the frequency of thrombophilia risk factors between patients who experienced thrombosis at/after AIHA diagnosis and those who did not. The distribution of prothrombotic risk factors was broadly similar. This was clear for inherited thrombophilia abnormalities detected in a greater proportion among patients without thrombosis (17/72, 24% *vs*. 3/20 15%). On the other hand, it should be noted that all seven patients with double or triple positivity for antiphospholipid antibodies experienced multiple thrombotic episodes, as well as thrombosis while on anticoagulants. Concerning conditions associated with AIHA, the patient with paroxysmal nocturnal hemoglobinuria experienced a massive pulmonary embolism during the diagnostic phase and without heparin treatment, while those with TE and myelodysplastic neoplasm had no thrombosis. No associations between pregnancy complications and thrombophilia during AIHA were noted.

Figure 1 shows thrombosis-free survival for the whole cohort. Over a cumulative observation period of 183,245 days (approximately 501.7 patient-years) the incidence of thrombotic events was 3.6 (95% confidence interval: 2.2-5.5) per 100 patient-years. The incidence was 10.6 (95% confidence interval: 2.6-18.6) per patient-years in patients without thrombophilia, and 6.7 (95% confidence interval: 0-19.2) per patient-years in those with thrombophilia.

Table 2. Thrombotic risk factors and thrombophilia abnormalities among patients with autoimmune hemolytic anemia.

Thrombotic risk factors	Thrombosis* during/after AIHA N=20	No thrombosis N=72	Total N=92
Prothrombotic risk factors before AIHA, N (%)			
OC use/pregnancy	4 (20)	22(30)	26 (28)
Previous cancer	2 (10)	12 (17)	14 (15)
Surgery/trauma	4 (20)	46 (64)	50 (54)
CVD risk factor**	5 (25)	35 (49)	40 (43)
Autoimmune diseases	2 (10)	19(26)	21 (23)
Personal history of VTE/ATE before AIHA, N (%)	4 (20)	4 (5)	10 (11)§
Family history of VTE/ATE before AIHA, N (%)	4 (20)	46 (64)	50 (54)§
Thrombophilia abnormalities, N (%)	3 (15)	17 (24)	20 (22)
AT, PC, PS deficiencies	1 (5)	5 (7)	6 (7)
Factor V Leiden	0	1 (1.4)	1 (1)
Prothrombin G20210A mutation	0	1 (1.4)	1 (1)
Lupus anticoagulant, anticardiolipin and/or anti-β2 antibodies	2 (10)	8 (11)	10 (11)†
Multiple defects	0	2 (3)	2 (2)‡

Screening included evaluation of inherited (antithrombin, protein C, and protein S levels, factor V Leiden, factor II G20210A mutation) and acquired forms (antiphospholipid antibodies, namely positivity for the lupus anticoagulant, anticardiolipin antibodies IgG/IgM, and anti-β2 glycoprotein I antibodies IgG/IgM). *Venous or arterial thrombotic events. **At least one comorbidity among hypertension, dyslipidemia and diabetes. §Six patients had both personal and family histories of venous/arterial thrombotic event before the first episode of autoimmune hemolytic anemia. †Five were triple-positive, two were double-positive, two had lupus anticoagulant, one was positive for anticardiolipin antibodies. ‡One patient had deficiencies of protein C plus antithrombin, one had factor V Leiden plus protein S deficiency. AIHA: autoimmune hemolytic anemia; OC: oral contraceptive; CVD: cardiovascular disease; VTE: venous thrombotic events; ATE: arterial thrombotic events; AT: antithrombin; PC: protein C; PS: protein S.

Online Supplementary Table S1 shows the analysis of the incidence of thrombotic events based on exposure to antithrombotic prophylaxis. Considering patients receiving prophylaxis (N=20), those receiving anticoagulation with vitamin K antagonists (N=13) accounted for 25.3 patient-years, during which two VTE and one ATE occurred, yielding incidence rates of 7.9 VTE and 3.9 ATE per 100 patient-years. Among those on antiplatelet therapy alone (N=5; 16.2 patient-years), one VTE was recorded (no ATE), with an incidence rate of 6.2 VTE per 100 patient-years. No thrombotic events were observed in the two patients who received combined vitamin K antagonist and antiplatelet therapy (2 patient-years). Regarding thrombophilia screening, 5/20 were positive, of whom two had a thrombotic event (1 VTE and 1 ATE); three had a prior history of thrombosis (1 had paroxysmal nocturnal hemoglobinuria).

Concerning patients not on prophylaxis (N=72), 11 VTE and 3 ATE occurred, corresponding to incidence rates of 2.4 VTE and 0.7 ATE per 100 patient-years. Notably, this group had a longer observation time of 458.1 patient-years compared with those receiving prophylaxis. Fifteen of these 72 patients, had a positive thrombophilia screening of whom only three had a thrombotic event (2 VTE and 1 ATE).

All in all, while thrombotic events were more frequent in the no-prophylaxis group in absolute terms, incidence rates appeared higher in patients receiving antithrombotic therapies, possibly due to the shorter observation period and a higher baseline thrombotic risk in the latter (incidence rate ratio=3.0; 95% confidence interval: 0.99-9.14).

In this study, we describe the presence of inherited or acquired thrombophilia in about 20% of patients with AIHA, the former accounting for about 7% and the latter being much more frequent. Our findings do not support a clear correlation between thrombotic events and positive thrombophilia screening, particularly for congenital risk factors (factors V and II mutations, natural anticoagulant deficiencies). The relationship with acquired risk factors is less clear-cut as it may be biased by their intrinsic time-dependent variability as well as by the time from the screening to the thrombotic event. There is great debate on the utility of thrombophilia screening in different settings, as recently outlined by the American Society of Hematology 2023 guidelines.[14] These mainly issued strong recommendations against screening, although with several exceptions, but did not consider hemolytic or autoimmune conditions.[14]

As a matter of fact, our findings support a possible effect of dual and triple positivity for antiphospholipid antibodies on the cumulative incidence of thrombosis. Consistently, antiphospholipid positivity was shown to be associated with thrombotic risk in large retrospective AIHA series and was present in two-thirds of patients experiencing thrombosis while on prophylaxis in this study.[5,10] Furthermore, the autoimmune nature of the disease further corroborates the utility of testing for antiphospholipid antibodies in clinical practice, as also suggested by the recent recommendations for Evans syndrome.[15] Altogether our findings support the decision to avoid screening for inherited thrombophilia in AIHA patients, while testing for acquired risk factors, particularly antiphospholipid antibodies, is advised. We would suggest that all patients be screened at diagnosis, and then those positive should be re-evaluated as per current guidelines (i.e., after 12 weeks).

Notably, AIHA remains the main trigger for thrombosis, with an incidence rate of 3.6 (95% confidence interval: 2.2-5.5) per 100 patient-years, and a strong association with hemolytic activity. In fact, of the 29 thrombotic events, 11 occurred during the first AIHA episode, and 12 during subsequent hemolytic relapses. Conversely, AIHA type and primary *versus* secondary form did not significantly

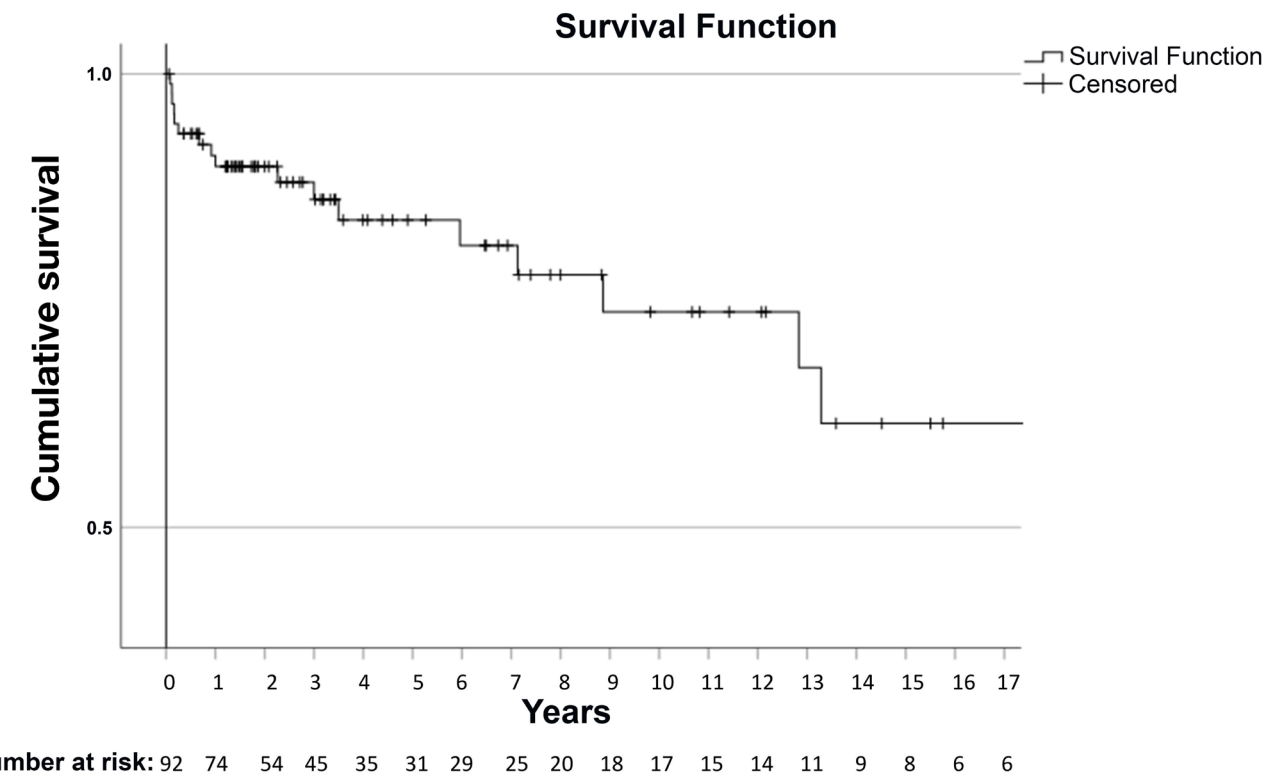

Figure 1. Thrombosis-free survival in patients with autoimmune hemolytic anemia. The cumulative incidence of thrombosis was analyzed by the Kaplan-Meier method. The exposure time was calculated as the time between the first episode of autoimmune hemolytic anemia to the venous/arterial thromboembolism or to the last follow-up for patients not experiencing thrombosis. In those patients receiving thromboprophylaxis the exposure time was calculated as the time from the start of prophylaxis to the thrombotic event/end of follow-up.

influence thrombotic risk; among diseases associated with AIHA, paroxysmal nocturnal hemoglobinuria was the only one associated with thrombosis, in line with the vicious thrombophilia observed in this disease.

Regarding anti-thrombotic prophylaxis, we observed a higher cumulative incidence of thrombosis in patients already on prophylaxis as compared to those not receiving prophylaxis. On the contrary, in a 2-year prospective cohort of 174 AIHA patients, no thrombotic events occurred in those who received low molecular weight heparin prophylaxis, while five events were observed in the group not receiving prophylaxis.[10] All in all, while prophylactic anticoagulation is recommended during hemolytic flares, prolonged anticoagulation during AIHA remissions should be evaluated on a case-by-case basis, mainly taking into account acquired risk factors. In this setting, age, cardiovascular risk factors, thrombophilic comorbidities, autoimmune diseases, and positivity for antiphospholipid antibodies may suggest long-term prophylaxis and inform a different AIHA therapeutic approach (i.e., avoiding splenectomy). The great uncertainty on the duration of anticoagulant prophylaxis is also outlined in the recent American Society of Hematology 2023 guidelines.[14]

The main limitations of this study are its retrospective design, the shorter follow-up while on anticoagulant prophylaxis, and the limited number of thrombotic events. Nonetheless, a significant strength lies in the comprehensive and systematic evaluation of thrombotic risk factors and outcomes in a large cohort of AIHA patients managed in a referral center.

In conclusion, routine congenital thrombophilia screening is not warranted in AIHA, while the evaluation of acquired and modifiable risk factors, particularly testing for antiphospholipid antibodies, is advisable. AIHA remains the major risk factor for thrombosis, warranting thromboprophylaxis during hemolytic events.

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Disclosures

No conflicts of interest to disclose.

Contributions

BF, NC, WB and MC designed the study, followed patients, wrote the manuscript and evaluated it for important intellectual content. MA performed the statistical analysis. CN, MB-A, GLP, MB, AA, JAG, FPe and FPa performed the clinical and laboratory evaluations of patients and revised the manuscript for important intellectual content.

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Data-sharing statement

All data have been included in the manuscript. Additional information may be obtained upon reasonable request to the corresponding author.

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