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Full Length Article

Bridging laboratory assays, genetics, and clinical phenotypes in antithrombin deficiency: Rethinking the diagnostic paradigm

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ABSTRACT

Background: Diagnosis of antithrombin deficiency (ATD), the most severe inherited thrombophilia, commonly relies on functional assays despite their uncertain sensitivity. Moreover, routine characterization of ATD remains uncommon due to limited supporting clinical data.
Objectives: This study aimed to evaluate the diagnostic sensitivity of commercial antithrombin (AT) activity assays and assess clinical differences among ATD types to refine the current diagnostic approach.
Methods: Eighty-eight patients with decreased AT activity and 124 consecutive patients with unprovoked venous thromboembolism were included. AT activity was measured using six assays. Genetic analysis of *SERPINC1* was performed by Sanger sequencing and multiplex ligation-dependent probe amplification; additionally, long-read whole-genome sequencing was conducted.
Results: Fifteen *SERPINC1* variants were detected, including two novel ones. AT Padua I (p.Arg79His) was the most prevalent (49%). Sensitivity varied across AT activity assays, particularly for types IIRS and IIHBS, with variant-specific discrepancies. AT Dublin (p.Val30Glu), causing transient deficiency, was undetected by all assays. Two assays demonstrated very high sensitivity (93%; $p < 0.001$), while two others showed poor sensitivity (46%). Regardless of measured AT activity, characterization of ATD type enabled better risk stratification. Type I was associated with early-onset and recurrent venous thromboembolism, and type IIHBS was distinctly linked to arterial thrombosis.
Conclusions: Assay sensitivity varies considerably, and only a few proved suitable as first-line tests. Genetic testing for common variants undetectable by even the most sensitive assays, along with ATD characterization, should be integrated into diagnostic algorithms. Our results also suggest that young patients with arterial thrombosis should be screened for ATD.

1. Introduction

Antithrombin deficiency (ATD) refers to a decreased natural anticoagulant, antithrombin (AT). Together with its physiological cofactor heparan sulfate or its pharmacological analogue heparin, AT primarily inhibits thrombin (FIIa) and factor Xa (FXa), the key enzymes in the coagulation cascade [1]. Consequently, ATD poses the highest risk for venous thromboembolism (VTE) among inherited thrombophilias [1,2], and is also associated with early and recurrent thrombosis [1,3]. Apart from VTE, arterial thrombosis (ATE) and pregnancy complications have been reported, however, the association remains debated due to limited evidence [3,4].

Congenital ATD is an autosomal dominant disorder, mostly caused by a genetic variant in the *SERPINC1* gene, which encodes AT [5]. Nearly all identified variants have deleterious consequences [5], which has contributed to the registration of 546 pathogenic variants to date [6]. These lead to two main ATD types. Type I (quantitative deficiency) results from nonsense, splicing, frameshift, structural, and some

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missense variants, all of which reduce plasma AT antigen levels [3,5]. While generally rare and occurring within individual families [3,7], type I carries a higher thrombosis risk [3,4]. In contrast, type II (qualitative deficiency) is predominantly caused by missense variants that do not affect antigen level but impair AT function due to defects in the reactive site (RS), heparin-binding site (HBS), or through pleiotropic effects (PE) [3,5]. Certain type II variants are more prevalent in specific populations, often due to the founder effect [8–11]. Although type II generally poses a lower thrombosis risk, this is largely attributed to type IIHBS, despite notable heterogeneity among individual variants [3,4]. Moreover, while homozygosity for ATD is typically lethal, rare cases of homozygous types IIHBS and IIRS have been reported, often resulting in severe clinical outcomes [3,4].

As all forms of ATD lead to diminished AT activity, functional assays are recommended as the first-line diagnostic test according to clinical guidelines [7] and supported by clinical practice. These are chromogenic assays that measure the inhibition of FIIa or FXa by endogenous AT in the presence of heparin. Analysis is typically conducted on automated coagulation analyzers with commercial reagent kits, which differ not only in the target protease but also in other assay conditions [7]. ATD is diagnosed when AT activity is below the reference range, generally set at 80% of normal plasma AT activity, though this threshold varies across laboratories [7]. Some assays have shown poor sensitivity in detecting ATD. FIIa-based assays tend to be more sensitive to type IIRS than those based on FXa inhibition [9,12–14], whereas certain FXa- or FIIa-based assays better detect type IIHBS [10,14–18]. Moreover, sensitivity may also depend on the underlying variant [14,16].

In addition to standard AT activity assays, other AT biochemical tests (antigen level and progressive activity) and molecular analyses are recommended [7] for ATD characterization, as VTE risk varies depending on ATD type and specific variant. Antigen measurement distinguishes type I from type II, while progressive activity (AT activity measured without added heparin) helps differentiate the less thrombogenic type IIHBS from types IIPE and IIRS. When available, molecular analysis can identify specific variants, including those missed by AT activity assays [7].

Recently, some experts have started to challenge the clinical value of AT activity assays [1,19], since their questionable sensitivity raises concerns that patients at high risk of thrombosis may go undetected. Given these limitations, genetic testing has been proposed as a more reliable first-line strategy [3,19,20]. At the same time, the limited sample size and diversity of documented ATD cases in studies weaken the evidence for clinically meaningful differences between ATD (sub) types. Genotype-based treatment is therefore not currently supported, and comprehensive ATD characterization is not widely available in clinical laboratories.

This study aimed to compare the sensitivity of six commercial AT activity assays across ATD types, employing the largest number of assays simultaneously evaluated to date. Additionally, the genetic background was explored using state-of-the-art genomic methods. With this approach, we aimed to expand the evidence on associations among laboratory phenotype, genotype, and clinical phenotype. Finally, the study evaluated how these findings could refine the ATD diagnostic approach.

2. Patients and methods

2.1. Patients

Patients who had at least one AT activity measurement below 80% recorded between 1991 and 2020 at the largest Slovenian university medical center were invited to provide an additional blood sample. This inclusion criterion was established to ensure that cases with only a single abnormal measurement who did not undergo repeat testing were not missed, and the 80% threshold was chosen as the general cut-off for suspecting ATD. During this period, AT activity was measured using either the Innovance or Berichrom assay (both Siemens Healthineers, Marburg, Germany). To identify potential cases with ATD that may not be detected by these assays, a control group of consecutive patients with unprovoked VTE before age 50, treated at the same center between 2010 and 2020, was also recruited. This group was selected due to its strong association with thrombophilia. Additionally, 20 healthy volunteers were included to determine the diagnostic performance of AT assays.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the National Medical Ethics Committee (0120–374/2020/3).

2.2. Clinical data

Clinical data were collected from the hospital information system and from questionnaires completed by patients.

Venous thrombosis, pulmonary embolism, and ATE were confirmed by appropriate objective methods. ATE was defined as myocardial infarction, ischemic stroke, transient ischemic attack, peripheral artery embolism, or acute limb ischemia. Recurrent pregnancy loss was defined as at least two fetal losses. Provoked VTE was recognized when a risk factor was present, as described previously [21].

2.3. Laboratory methods

2.3.1. Patient samples

Blood was drawn into 3.2% Na-citrate and EDTA tubes for hemostasis and genetic testing, respectively. Samples for hemostasis tests were centrifuged at 2500 x*g* for 15 min within four hours of sampling. Plasma was aliquoted and stored at −80 °C. Genomic DNA was isolated with a commercial kit Flexigen DNA (Qiagen, Hilden, Germany) according to the provided protocol and stored at 4 °C.

2.3.2. AT biochemical assays

AT activity was measured using six commercial AT activity assays. These were selected based on the type of enzyme used, enabling assessment of the enzymes' influence on assay sensitivity. Specifically, two human FXa-based, two bovine FXa-based, and two FIIa-based assays were included (Supplementary Table S1). Innovance (INNOVANCE® Antithrombin, Siemens Healthineers), Sta-Stachrom (STA-Stachrom AT III, Diagnostica Stago, Asnières, France), BioXaLRT (BIOPHEN™ AT anti-(h)-Xa LRT, Hyphen BioMed, Neuville-Sur-Oise, France), and BioIIa (BIOPHEN™ Antithrombin (Anti-IIa), Hyphen BioMed) assays were performed on the Sysmex CS-2500 analyzer (Siemens Healthineers). The Hemosil assay (HemosIL Liquid Antithrombin®, Werfen, Bedford, USA) was conducted on the ACL TOP analyzer (Werfen). The BioXa assay (BIOPHEN™ Antithrombin, Hyphen BioMed) was run manually using a microplate spectrophotometer (Sunrise™, Tecan).

Antigen levels were measured using the Liatest AT III kit (Diagnostica Stago). Progressive activity was determined using an adapted heparin cofactor–based method (BIOPHEN™ Antithrombin, Hyphen BioMed), in which the standard heparin-containing buffer was replaced with buffer without it (AT-Tris Buffer-Anti Xa, Progressive Antithrombin, Hyphen BioMed). Both tests were performed using a microplate spectrophotometer.

Innovance, BioXaLRT, and Hemosil reagents, together with the analyzers on which they were used, comply with Regulation (EU) 2017/746 on in vitro diagnostic medical devices (IVDR) [22]. Sta-Stachrom and BioIIa protocols, originally intended for STA analyzers (Diagnostica Stago), were adapted for use on the CS-2500 analyzer. All other assays that are not IVDR-certified were performed according to the manufacturer's protocols. Assays were calibrated using kit calibrators traceable to the WHO standard. Reference ranges stated by the manufacturer were verified and confirmed using samples from 20 healthy volunteers.

Samples were thawed at 37 °C for 5 min in a thermoshaker. Automated measurements were performed on a single aliquot in batches of

ten samples, while separate aliquots were used for manual activity and antigen measurements. The reliability of the results was ensured by running quality control material at the beginning of each series and after each reagent change.

Screening coagulation assays (prothrombin time, activated partial thromboplastin time, thrombin time, D-dimer, fibrinogen) and anti-Xa were performed to assess sample integrity and to exclude acquired ATD resulting from anticoagulant therapy. Samples from patients receiving direct oral anticoagulants (DOACs) were treated with DOAC-Stop™ (Haematex Research, Sydney, Australia). Drug neutralization was confirmed by anti-Xa for rivaroxaban, apixaban, and edoxaban, and by thrombin time for dabigatran before AT activity measurement. None of the patients had been taking low-molecular-weight heparin. Patients with acquired ATD due to predefined clinical conditions [7] were excluded.

2.3.3. Molecular genetic analysis

Genetic analysis of *SERPINC1* was performed using Sanger sequencing of all seven exons and flanking regions on the ABI Prism® 3500 (Applied Biosystems, Waltham, USA) (Supplementary Method S1). For patients with no variant identified but a type I laboratory phenotype, copy number variations were investigated using multiplex ligation-dependent probe amplification (MLPA) with the SALSA MLPA Kit P227 *SERPINC1* (MRC Holland, Amsterdam, the Netherlands), following the manufacturer's instructions.

When no variant was detected after these analyses but a strong clinical indication of ATD was present (type I phenotype and unprovoked VTE, or decreased AT activity across all assays), whole-genome sequencing was performed using nanopore technology on the PromethION (Oxford Nanopore Technologies, Oxford, UK) (Supplementary Method S2). Variants were assessed in *SERPINC1* and 16 additional genes associated with reduced AT activity according to Human Phenotype Ontology (HPO; HP:0001976).

Nucleotide changes were annotated relative to the *SERPINC1* genomic reference sequence (NG_012462.1), and coding variants were described according to the transcript reference sequence (NM_000488.4) using Human Genome Variation Society (HGVS) nomenclature. Pathogenicity was classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines [23], applying gene-specific criteria from the ClinGen Thrombosis Variant Curation Expert Panel (VCEP) for the *SERPINC1 gene*.

The laboratory workflow for different cohorts is presented in Fig. 1.

2.3.4. ATD diagnosis and characterization

ATD was diagnosed when a causative genetic variant was detected, or when decreased AT activity was determined with at least one of the tested assays according to manufacturer-provided reference ranges. Previously uncharacterized variants were assigned to ATD subtypes based on antigen levels and progressive activity. In ambiguous cases, characterization was guided by predicted effects on AT structural stability using FoldX (v5.1) (FoldX Consortium, Barcelona, Spain) (Supplementary Method S3).

2.3.5. Other plasma assays

To exclude additional thrombophilic factors contributing to VTE, other types of thrombophilia, including protein C and protein S deficiencies, resistance to activated protein C, factor V Leiden (FVL), prothrombin G20210A and lupus anticoagulants, were screened.

In patients with genetic variants associated with AT glycosylation abnormalities, the activity of coagulation factors (FII, FX, FVII, FXI, FIX) was measured to support the presence of congenital disorders of glycosylation (CDG) [24].

2.4. Statistics

Data normality was evaluated using the Shapiro-Wilk test. Since most variables followed a non-normal distribution, results were presented as median and range (min–max).

Differences in AT biochemical assay results between ATD types were tested using the Mann-Whitney *U* test. To determine the diagnostic specificity and sensitivity of the AT activity assays, healthy volunteers were considered true negatives, while patients with a *SERPINC1* variant or familial ATD without an identifiable variant were considered true positives. Diagnostic performance of the assays was assessed by ROC analysis, with determination of optimal cut-off values and comparison of AUCs using the DeLong test. Differences in sensitivity across assays were evaluated using Cochran's Q test, followed by McNemar's test with Bonferroni correction.

Kaplan–Meier analysis with the Log-Rank test was used to assess differences between ATD types in the risk of a first thrombotic event,

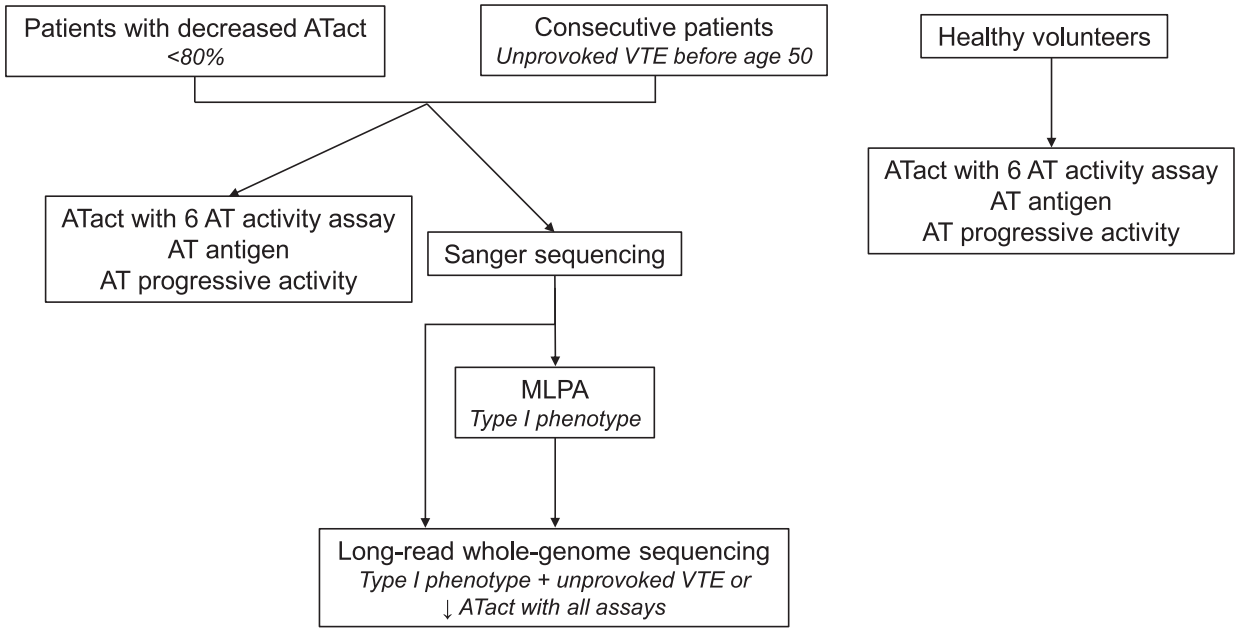

Fig. 1. The laboratory workflow for different cohorts. AT, antithrombin; ATAct, antithrombin activity; VTE, venous thromboembolism.

defined either as a composite of VTE and ATE or as VTE alone. Differences in the number of thrombotic events between types were evaluated using Poisson regression, adjusted for age and sex. Differences in the frequency of VTE, unprovoked VTE, ATE, and miscarriages between types, as well as the association between ATD (type) and ATE, were analyzed using the Chi^2 test or Fisher's exact test when expected frequencies were not met.

The association between AT activity (measured by Innovance and Sta-Stachrom assays) and VTE recurrence and thrombosis localization was assessed by comparing AT activity between groups using the Mann–Whitney *U* test and by determining optimal thresholds via ROC analysis.

Statistical significance was set at $p < 0.05$. Analyses were performed in SPSS (IBM, Armonk, USA), except ROC analyses, which were conducted in MedCalc (MedCalc Software Ltd., Ostend, Belgium).

3. Results

3.1. Patient characteristics

A total of 212 patients were enrolled, including 88 patients (78 index families) with previously documented decreased AT activity (<80%) and 124 consecutive patients (Table 1). In the first cohort, ATD was diagnosed in 62 patients, with genetic confirmation or familial ATD in 42. Among the 124 consecutive patients, 37 (30%) had thrombophilia. ATD was diagnosed in five of these patients, with a genetic cause identified in four (3% of the consecutive patients). Overall, 67 patients had ATD, including 43 with genetically confirmed ATD and three relatives with decreased AT activity but no identifiable genetic variant (Table 2).

Among the 67 ATD patients, 39 (58%) developed VTE only, with 18 (27%) experiencing unprovoked VTE before age 50 (Table 2). ATE occurred exclusively in 27% of patients, and miscarriages in 4%. One index patient and two unrelated family members were asymptomatic (two females, one male; median age 53 years, range 26–55). Six patients with *SERPINC1* variants (five type II, one causing transient ATD) also had another thrombophilic defect.

3.2. Genetic background

A *SERPINC1* variant was identified in 66% of ATD patients (43/67), including 61% of index cases (35/57). Among 19 ATD patients who lacked genetic confirmation and family history data, five showed decreased AT activity across all assays; two of these patients showed borderline-normal activity with BioXa, which demonstrated poor diagnostic performance (discussed later). We therefore considered these five patients true positives for congenital ATD (Supplementary Table S10).

Table 1
Demographic data of study participants.

	Decreased ATAct	Consecutive
Number of patients (index)	88 (78)	124 (124)
Age, years (range)	52 (22–80)	47 (21–61)
Sex (F/M), N	49/39	34/90
ATD (genetically confirmed or familial ATD/ decreased AT activity), N	42/62	4/5
Thrombophilic defect/concomitant thrombophilic defect, N	69/8	37/2
Symptomatic, N	80	124
VTE (isolated/composite), N	46/4	120/4
Unprovoked VTE, <50 years (isolated/ composite), N	19/2	120/4
ATE (isolated/composite), N	25/3	0/2
Pregnancy loss (isolated/composite), N	5/3	0/2

Abbreviations: ATAct, antithrombin activity; ATE, arterial thrombosis; F, female; M, male; N, number; VTE, venous thromboembolism.

Excluding the remaining 14 patients increased the variant detection rate to 81% (43/53). The detection rate was highest in patients with unprovoked VTE before age 50 (71%, 15/21), followed by those with ATE (65%, 13/20), and lowest in late-onset or provoked VTE (55%, 12/22).

Fifteen different heterozygous variants in the *SERPINC1* gene were identified (Table 3), two of which (c.196G > T, p.Glu66Ter and c.1061del, p.Arg354ProfsTer10) had not been previously reported. In addition to single nucleotide variants (eight missense, five nonsense, and one deletion), there was one structural variant, a whole-gene deletion. Most patients were type II carriers (71% of index cases), while type I variants were present in 23%. Two patients (6%) carried the c.89 T > A (p.Val30Glu, AT Dublin) variant, which causes transient deficiency and was identified only in consecutive patients. The most common variant in our population was c.236G > A (p.Arg79His, AT Padua I) (49%), followed by c.218C > T (p.Pro73Leu, AT Basel) (11%) and c.391C > T (p.Leu131Phe, AT Budapest III) (3%), all causing type IIHBS. Two patients carried the type IIPE variant, and one carried c.1277C > T (p.Ser426Leu, AT Denver), a type IIRS variant. Each of the eight type I variants was found in a single family.

In two patients, decreased AT activity resulted from CDG, representing 4% of ATD cases. The first had heterozygous variants in *PMM2* and *ALG6* genes, and the second in the *ALG12* gene. Although these variants typically cause disease only in the homozygous form, reports link a single defect combined with alcohol abuse to decreased AT levels [34], which could explain these cases. Both patients also had decreased levels of FXI and protein C, supporting the presence of CDG [24] (Supplementary Table S11).

3.3. Genotype–laboratory phenotype

AT activity was decreased in all variants except AT Dublin, and was lower in type I than in type II across all assays. Antigen levels were reduced in all type I cases. Among type II variants, antigen levels were generally normal, except for a mild decrease in type IIPE and a more pronounced reduction in one type IIHBS variant, AT Budapest III. AT Dublin showed normal antigen levels. Progressive AT activity was normal in type IIHBS, as expected, but also in one patient with a type IIPE variant and borderline-normal in type IIRS. AT Dublin also showed normal progressive activity, whereas it was decreased in type I. The five patients, suspected of having congenital ATD based on uniformly decreased activity across assays, had low antigen levels and borderline progressive activity, consistent with type I or IIPE deficiency (Table 4; for detailed individual variant–laboratory phenotype associations, see Supplementary Table S12).

Comparison of AT activity assays across a total of 212 samples showed consistent results in 172 (81%), with 147 classified as normal and 25 as decreased, while 40 samples were discrepant.

Focusing on 46 cases with genetically confirmed or familial ATD, significant differences in sensitivity among assays were observed (Fig. 2). Innovance and Sta-Stachrom were the most sensitive assays, with a sensitivity of 93% ($p \leq 0.002$ vs. all other assays). BioXaLRT and BioIIa showed lower sensitivities (67%), but still performed significantly better than Hemosil and BioXa, which had sensitivities of 46% ($p = 0.002$ vs. BioXaLRT and BioIIa) (Fig. 2A).

None of the assays detected AT Dublin, while all demonstrated 100% sensitivity to type I. Sensitivity to type II varied significantly among assays (Fig. 2B), particularly for the type IIRS variant (AT Denver) and type IIHBS ($p < 0.001$) (Fig. 2C), with additional variations among specific type IIHBS variants. Sensitivity to AT Padua I and AT Basel differed significantly across assays ($p < 0.001$ and $p = 0.006$, respectively), whereas all assays detected AT Budapest III (Fig. 2D).

For the most sensitive assays, Innovance and Sta-Stachrom, variant detection was most likely when AT activity was below 70%; in this range, over 80% of patients carried a *SERPINC1* variant. In the borderline activity range (70–80%), which often poses the greatest diagnostic

Table 2
Characteristics of patients with ATD.

	ATD (total)	ATD confirmed by a *SERPINC1* variant	Familial ATD[a]	ATD due to CDG	Decreased ATAct without a detected genetic variant and unknown familial ATD
Number of patients (index)	**67 (57)**	43 (35)	3 (1)	2 (2)	19 (19)
Age, years (range)	**52 (22–77)**	45 (22–66)	48 (41–69)	55–66	57 (44–77)
Sex (F/M), N	**31/36**	24/19	1/2	0/2	5/14
Concomitant thrombophilic defect, N	**8**	6	0	0	2
Symptomatic, N	**64**	41	3	2	18
VTE (isolated/composite), N	**39/4**	24/3	3/0	0/0	12/1
Unprovoked VTE, <50 years (isolated/composite), N	**18/3**	13/2	2/0	0/0	3/1
ATE (isolated/composite), N	**18/2**	12/1	0/0	2/0	4/1
Pregnancy loss (isolated/composite), N	**3/2**	2/2	0/0	0/0	1/0

Abbreviations: ATAct, antithrombin activity; ATD, antithrombin deficiency; ATE, arterial thrombosis; CDG, congenital disorders of glycosylation; F, female; M, male; N, number; VTE, venous thromboembolism.
[a] Family members without a detected genetic variant but clearly displaying a type I laboratory phenotype (with equally decreased AT activity and antigen levels below 50%).

Table 3
SERPINC1 variants in the study population.

ATD type	Nucleotide change	Amino acid change	Method of detection	Pathogenicity	ACMG codes	dbSNP/ClinVar ID	Number of patients, total (index)	Literature reference
Type I	c.80G > A	p.Trp27Ter	Sanger	LP	PVS1, PM2_sup, PP4	NA/ NA	1 (1)	[25]
	c.196G > T	p.Glu66Ter	Sanger	LP	PVS1, PM2_sup, PP4	NA/ NA	1 (1)	novel
	c.374G > A[a]	p.Gly125Asp	Sanger	VUS	PP3, PM5_sup, PM2_sup, PS4_mod	NA/ 2,907,297	3 (1)	[10,26,27]
	c.607C > T	p.Gln203Ter	Sanger	P	PVS1, PM2_sup, PS4_mod	rs2102785788/ NA	4 (1)	[26,28]
	c.685C > T	p.Arg229Ter	Sanger	LP	PVS1, PM2_sup, PP4	rs1657743081/ NA	1 (1)	[14,19]
	c.1061del	p.Arg354ProfsTer10	Sanger	P	PVS1, PM2_sup, PS4_mod	NA/ NA	2 (1)	novel
	c.1171C > T	p.Arg391Ter	Sanger	P	PVS1_str, PP1_str, PM2_sup PS4_mod, PP4	NA/ 2,734,038	1 (1)	[10,17,28]
	Whole-gene deletion	/	MLPA, Nanopore	P	2A; 1 pt[b]	/	2 (1)	[14,17]
Type IIPE	c.1063 T > G[a]	p.Phe355Val	Sanger	VUS	PM2_sup, PP3, PP4	rs1035744320/ 2,627,762	1 (1)	[25]
	c.1204 A > G[a]	p.Lys402Glu	Sanger	VUS	PM2_sup, PP3, PP4	rs2526559490/ 3,018,042	1 (1)	NA
Type IIRS	c.1277C > T	p.Ser426Leu (AT Denver)	Sanger	P	PP3, PM2_sup, PS3_sup, PP1_str, PS4, PP4	rs121909550/ 18,010	1 (1)	[14,20]
Type IIHBS	c.218C > T	p.Pro73Leu (AT Basel)	Sanger	P	PM1, PS4, PP4, PP3, PP1_str,	rs121909551/ 18,011	4 (4)	[8,10,14,16–18,25,26,29–31]
	c.236G > A	p.Arg79His (AT Padua I)	Sanger, Nanopore	P	PP3, PM1, PP1_str, PS4, PP4	rs121909552/ 18,014	18 (17)	[8,14,16,17,26–28]
	c.391C > T	p.Leu131Phe (AT Budapest III)	Sanger	P	PP3, PS4, PP1_str	rs121909567/ 18,034	1 (1)	[8,14,25–28,32,33]
Transient	c.89 T > A	p.Val30Glu (AT Dublin)	Sanger	P	PS3, PS4, PP1_mod	rs2227624/ 18,012	2 (2)	[14,31]

Abbreviations: ACMG, American College of Medical Genetics and Genomics; AT, antithrombin; LP, likely pathogenic; P, pathogenic; mod, moderate evidence; NA, not available; str, strong evidence; sup, supporting evidence; VUS, Variant of Uncertain Significance.
[a] The ATD type of variant was determined based on its predicted impact on structural stability, using FoldX software.
[b] ACMG/ClinGen standards for copy number variation interpretation.

and therapeutic challenges, the detection rate remained high for Sta-Stachrom (79%), whereas AT activity measured by Innovance within the same range was rarely associated with a genetic variant (9%) (Fig. 3).

Innovance, Sta-Stachrom, BioXaLRT, and BioIIa demonstrated excellent discriminatory performance (AUC $\geq$ 0.90), with no significant differences observed between them ($p \geq 0.120$). Hemosil (AUC = 0.76) and the manual BioXa assay (AUC = 0.52, $p = 0.775$) showed significantly lower AUC (both $p < 0.001$), with the latter demonstrating no discriminatory ability. Cut-off adjustments improved sensitivity for all

Table 4
AT activity determined by all six assays, progressive activity and antigen levels in patients with ATD. Medians (min – max) are shown.

		Type I	Type II	AT Dublin	IIPE	IIRS	IIHBS	AT Padua I	AT Basel	AT Budapest III	Undetected genetic variant[a]
N		18[b]	26	2	2	1	23	18	4	1	5
ATAct (%)	**Innovance**	52 (41–55)	56 (50–82)[c]	87–97	65-82	76	55 (50–64)	55 (50–60)	57 (54–64)	55	70 (59–72)
	Sta-Stachrom	52 (43–56)	71 (58–84)[c]	94–108	68-78	58	71 (62–84)	71 (62–76)	73 (68–84)	62	75 (56–78)
	BioXaLRT	51 (39–56)	85 (63–99)[c]	98–99	63-73	99	85 (65–97)	87 (65–97)	79 (77–91)	66	73 (59–78)
	BioIIa	51 (41–56)	80 (62–96)[c]	91–95	62-77	63	81 (73–96)	80 (73–89)	88 (86–96)	74	70 (54–72)
	Hemosil	44 (26–48)	111 (57–136)[c]	94–102	57-72	111	113 (77–136)	112 (89–124)	121 (111–136)	77	65 (51–77)
	BioXa	51 (26–60)	91 (64–106)[c]	99–107	64-76	95	91 (75–106)	91 (83–106)	94 (86–103)	75	77 (59–81)
ProgAct (%)		70 (59–84)	121 (77–135)[c]	104–109	77-95	81	122 (94–135)	123 (108–135)	117 (94–126)	107	80 (77–86)
Ag (%)		48 (41–63)	93 (65–107)[c]	88–100	74-75	99	93 (65–107)	93 (84–107)	96 (93–98)	65	74 (63–83)

Abbreviations: Ag, antigen; AT, antithrombin; ATAct, antithrombin activity; N, number; ProgAct, progressive activity.
Note: Lower value of reference range: Innovance <79%, Sta-Stachrom <80%, BioXaLRT <85%, BioIIa <80%, Hemosil <83%, BioXa <80%.
[a] Patients considered as true positives for congenital ATD, who lacked genetic confirmation and family history data, but showed decreased AT activity across all assays; two had borderline-normal results with BioXa, which performed poorly.
[b] Including three family members with no detected genetic variant but clearly displaying a type I laboratory phenotype (with equally decreased AT activity and antigen levels below 50%).
[c] $p < 0.05$ compared to type I.

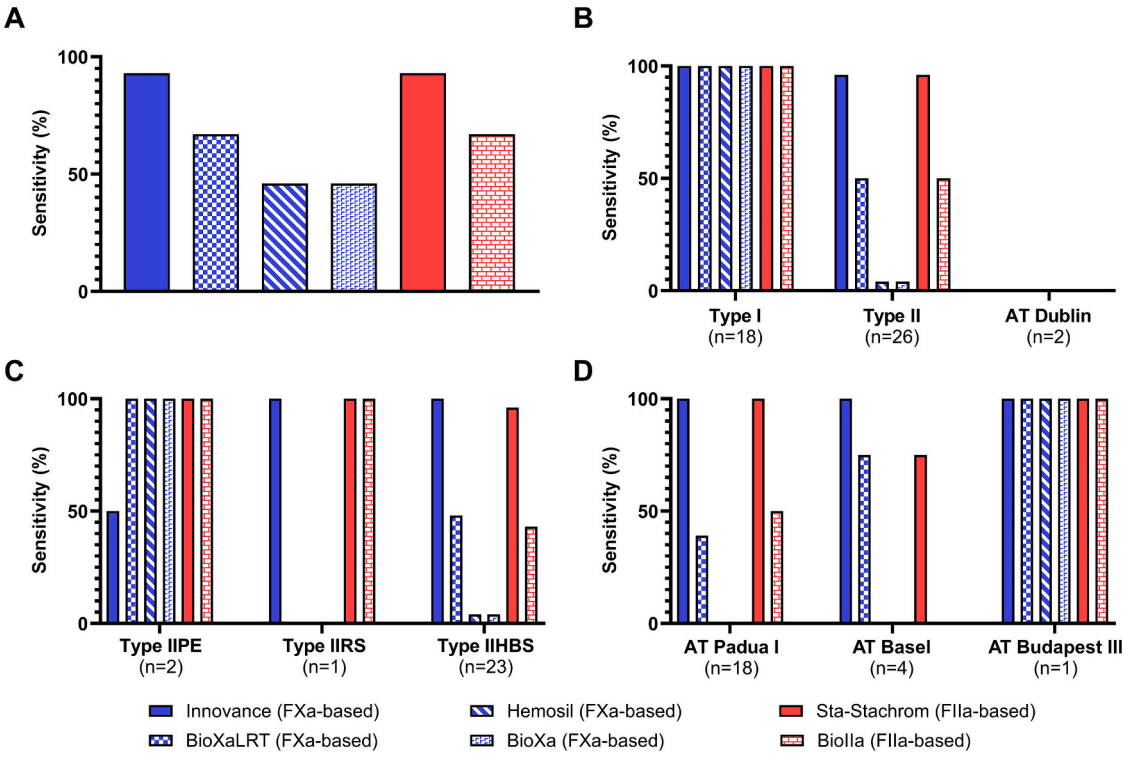

Fig. 2. Sensitivity of AT activity assays to: (A) genetically confirmed or familial ATD cases; (B) ATD types and the variant causing transient ATD (AT Dublin); (C) type II subtypes; and (D) IIHBS variants.

assays, with only a minor loss in specificity for BioXaLRT and BioIIa and complete loss of specificity for BioXa. Despite these adjustments, AT Dublin became detectable only by BioXaLRT and, in some cases, by Innovance (Supplementary Table S13).

3.4. Genotype–clinical phenotype

Patients with type I ATD developed their first thrombotic event significantly earlier than those with type II. This applies to both the composite of VTE and ATE (23 vs. 40 years, $p = 0.006$) and to VTE alone (22 vs. 39 years, $p < 0.001$). Both patients with AT Dublin had a similar onset to those with type II. The single patient with type IIRS developed the first event much earlier than other patients with type II. In the IIHBS cohort, no substantial difference in age at first event was observed between carriers of AT Padua I and AT Basel, whereas the single patient with AT Budapest III was notably younger (Fig. 4A).

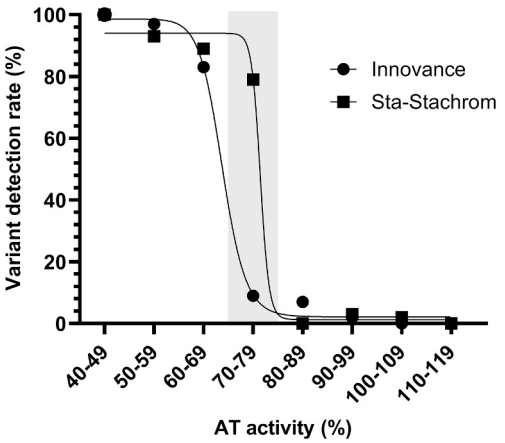

Fig. 3. Variant detection rate in relation to AT activity measured with two assays. Borderline AT activity values (70–80%) are shaded in grey.

Patients with type I also experienced more thrombotic events than those with type II, with a significantly higher risk of VTE (OR 2.99; 95% CI: 1.60–5.56; $p = 0.001$), while the increased risk for composite thrombotic events was not significant ($p = 0.055$). Patients with AT Dublin developed the same number of thrombotic events as those with type II. Among patients with type II, both patients with type IIPE had the highest number of events, exceeding even those with type I. Event frequencies were similar across carriers of type IIHBS variants (Fig. 4B).

Unprovoked VTE occurred more frequently in type I (69%) than in type II (58%); however, this difference was not significant ($p = 0.698$). The only patient with type IIPE who developed VTE and the single type IIRS patient experienced unprovoked events. In contrast, VTE was unprovoked in only half of patients with type IIHBS, mainly reflecting AT Padua I carriers, as only one patient with AT Basel and the single patient with AT Budapest III experienced VTE (Fig. 4C).

Most patients with type I (89%) developed VTE, while only 11% experienced ATE. In contrast, VTE and ATE occurred in similar proportions among patients with type II, at 46% and 42%, respectively. Consequently, patients with type I had a significantly higher risk of developing VTE compared to those with type II (OR 9.33; 95% CI: 1.78–49.08, $p = 0.004$), whereas patients with type II had an increased risk of ATE (OR 5.87; 95% CI: 1.11–30.95, $p = 0.026$) compared to type I group. Overall, the presence of ATD increased the risk of ATE by 3.45-fold (95% CI: 1.53–7.80; $p = 0.002$), mainly driven by type II (OR 6.43; 95% CI: 2.55–16.22; $p < 0.001$), with no significant increase in risk observed for type I ($p = 1.000$) when compared with the remaining patients included in the study (i.e., all patients without genetically confirmed or familial ATD). ATE was particularly observed in patients with type IIHBS, with AT Basel carriers showing the highest proportion of ATE. It was also present in AT Padua I carriers, but in similar proportion to VTE. Pregnancy loss occurred in two women with type I (20%) and two with type II (12%), with no significant difference between groups (p = 1.000) (Fig. 4D).

Long-term anticoagulation was received by 54% of genetically confirmed or familial ATD patients who developed a thrombotic event, with a higher proportion in those with type I (71% in type I vs. 41% in type II). This difference largely reflects the higher number of patients with recurrent VTE in the type I group, since among those with a single VTE event, the proportions of patients on anticoagulant therapy were similar between type I and type II (50% vs. 40%). Clinical characteristics by ATD type and variant are detailed in Supplementary Table S14 and Supplementary Table S12, respectively.

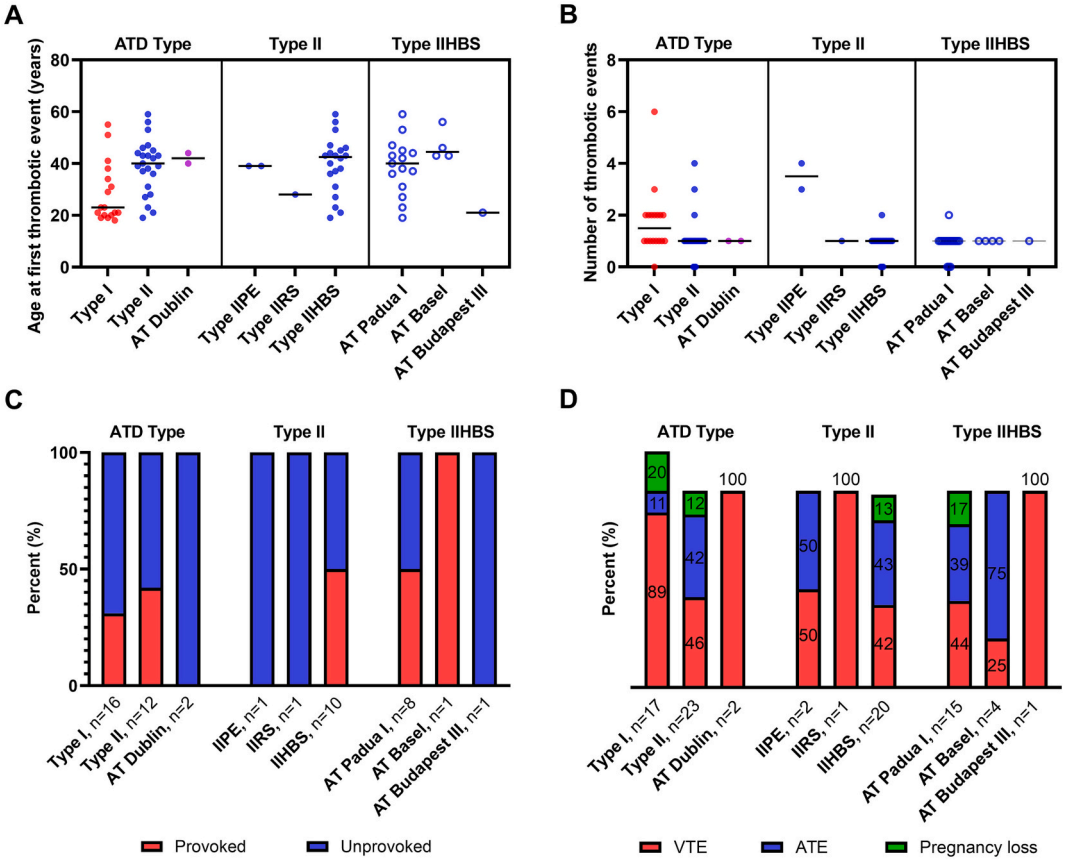

Fig. 4. Genotype–clinical phenotype correlation. Differences across ATD (sub)types and variants in: (A) age at first thrombotic event; (B) number of thrombotic events; (C) frequency of (un)provoked VTE; and (D) clinical manifestations.

3.5. Laboratory–clinical phenotype

A significant difference in AT activity between patients with and without recurrent VTE was observed using either of the two most sensitive assays (Innovance: $p = 0.022$; Sta-Stachrom: $p = 0.002$). For identifying patients with recurrence, Sta-Stachrom demonstrated good discriminatory performance (AUC = 0.82, 95% CI: 0.67–0.92, $p = 0.0001$) at a threshold of ≤52% while Innovance showed moderate discrimination (AUC = 0.73, 95% CI: 0.58–0.86, $p = 0.015$) at ≤53%. Within the 70–80% AT activity range, Sta-Stachrom identified only one patient with recurrence (10%) and 47% of those without recurrence, while Innovance detected one patient without recurrence in this range (Fig. 5A).

Both assays also distinguished VTE from ATE, with AUCs of 0.75 (Innovance) and 0.79 (Sta-Stachrom) at respective thresholds of ≤53% and ≤ 55%. The borderline range was more commonly associated with ATE (58% of ATE cases vs. 22% of VTE cases) when measured with Sta-Stachrom, whereas one VTE patient was identified in this range when measured with Innovance (Fig. 5B).

Although Innovance reached statistical significance, the actual difference in AT activity between groups was minimal and likely within the assay's measurement error, limiting its clinical utility.

4. Discussion

This study evaluated the diagnostic performance of commercial AT activity assays, encompassing the largest set of FXa- and FIIa-based assays across all ATD types to date. We explored the genetic background of ATD by complementing standard diagnostics with long-read whole-genome sequencing to ensure comprehensive variant detection. By selecting patients solely based on decreased AT activity, we likely captured all relevant clinical manifestations. Finally, we examined the relationships among genetic variants, laboratory findings, and clinical presentation to inform refinements to the current diagnostic algorithm.

The genetic background of our cohort is heterogeneous, with two novel variants identified. However, AT Padua I is the predominant variant (49% of index cases). Its estimated prevalence in Slovenia (0.01–0.1%) may exceed ~0.018% reported in European populations [35] and, since it is not predominant elsewhere in Europe [16,17,27–29,36], this strongly suggests a founder effect. Interestingly, AT Budapest III, a founder variant in Hungary [8] and commonly found in other European countries [25–28,32,33], was identified in only one of our studied patients, highlighting the unpredictability of the genetic background despite geographic proximity.

In our study, 81% of cases with decreased AT activity were explained by *SERPINC1* variants, consistent with detection rates of 63–100% reported in other studies [10,17,18,20,25,27,29,33,36]. Although assay-dependent, the detection rate remained high even at nearly normal AT activity (as also reported [33]), indicating that mild activity reductions can still be clinically relevant. The detection rate was not improved by long-read sequencing in our study, in contrast to previous reports [37,38]. This underscores that even comprehensive sequencing cannot fully explain ATD. The unresolved cases suggest additional pathogenic mechanisms, including defects in genes involved in N-glycosylation, which were also observed in our cohort at expected frequencies of up to 5%. This points to considering CDG in patients with a type I phenotype, which requires distinct treatment. Other mechanisms, including epigenetic changes, remain largely unexplored [39], mainly due to tissue-specific methylation [40]. AT also has many non-anticoagulant roles [3], implying protein interactions that may influence its activity and merit further study.

Our results also show that functional assays cannot detect all patients carrying a *SERPINC1* variant, consistent with previous reports [10,14–17,41]. As in other studies, sensitivity varied markedly between assays, which was largely limited to types IIRS and IIHBS, with variant-specific differences. AT Padua I and AT Basel were harder to detect than AT Budapest III, whose milder quantitative deficiency [16,42] was confirmed by our measurements and likely explains its more reliable detection. Using multiple FIIa- and FXa-based assays allowed us to assess the influence of enzyme type on diagnostic sensitivity. Detection of type IIHBS variants appears enzyme-independent, in agreement with a recent study [14] that also evaluated a broad range of assays based on different enzymes and with a meta-analysis [43]. Specifically, both Sta-Stachrom and Innovance showed high sensitivity to all type IIHBS variants despite using different enzymes, whereas Hemosil and Berichrom (the latter not included in our study), which also use distinct enzymes, showed poor sensitivity. Although Sta-Stachrom exhibited poor specificity in the comparable study [14], it demonstrated 100% specificity at the same cut-off in our study. This likely reflects analyzer-related differences, particularly incubation time, identified as a key factor affecting sensitivity [14]. However, detailed analysis of all assay characteristics is constrained by confidential manufacturer data.

In contrast, sensitivity to type IIRS variants seems more dependent on the enzyme used. All FIIa-based assays detected type IIRS variant in our study (Sta-Stachrom and BioIIa detected AT Denver) and in the comparable study (Sta-Stachrom and Berichrom detected AT Cambridge II (c.1246G > T, p.Ala416Ser)) [14], whereas FXa-based assays showed variable sensitivity. Specifically, BioXaLRT detected AT Cambridge II but not AT Denver, whereas Innovance detected AT Denver but not AT Cambridge II, and Hemosil failed to detect either variant. These findings indicate that FIIa-based assays are generally more sensitive for IIRS variants, while the performance of FXa-based assays depends on the variant and assay characteristics.

Variants causing transient ATD, such as AT Dublin, are particularly

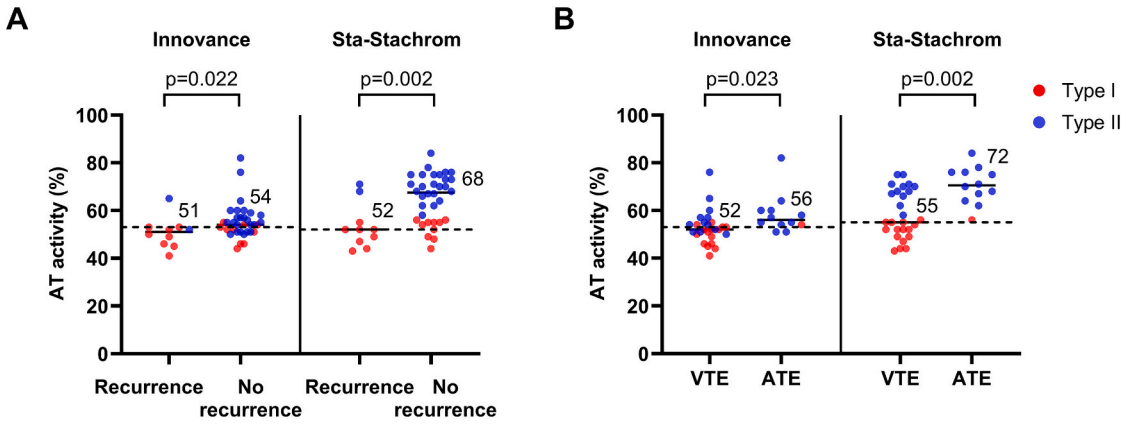

Fig. 5. Comparison of AT activity between assays in (A) patients with and without recurrent VTE, and (B) patients with VTE and ATE. Dashed lines indicate thresholds.

difficult to detect because their pathogenic mechanism makes detection highly dependent on the timing of sampling [44]. This is most probably the reason for the differing sensitivities of the same assays across studies. In our study, AT Dublin was undetected by all assays, whereas in another study [14] it was detected by the same assays, except Innovance. Moreover, when detected, it may present as either type I [26,27] or type II [10,27], reflecting its complex laboratory phenotype.

Beyond the laboratory phenotype, ATD type influenced clinical presentation. Consistent with previous reports [17,26,30], type I showed a more severe form of ATD with earlier thrombotic events and a higher risk of recurrence compared to type II, and especially compared to type IIHBS. Type I was also associated with early-onset VTE, whereas type II had a higher prevalence of ATE, typically occurring later, but still before age 45, as also observed previously [17,30,41]. This likely reflects lifelong anticoagulation in patients with type I after their first VTE, which may reduce ATE occurrence later in life. Variant-specific mechanisms might also contribute. ATE was more frequent among certain IIHBS variants, particularly AT Basel and, to a lesser extent, AT Padua I, consistent with earlier reports [10,16,17,30]. In addition AT Cambridge II is known to be significantly associated with ATE [45]. These findings indicate that ATD also confers a risk for ATE and suggest that conflicting results in meta-analyses [46,47] may stem from the lack of subtype or variant stratification. In line with these clinical differences, we also observed a higher proportion of long-term anticoagulation among patients with type I ATD. Although our data on anticoagulant therapy do not allow conclusions about the independent effect of genotype on anticoagulation strategy, they are consistent with the more severe thrombotic phenotype of type I and suggest that different ATD subtypes may warrant distinct clinical considerations.

We showed that the predictive value of AT activity for VTE recurrence or thrombosis localization depends on the assay employed. Between the two most sensitive assays, only Sta-Stachrom effectively identified patients with recurrence. Its optimal threshold was well below the 70–80% AT activity range previously associated with recurrence [48]. This discrepancy may be due to assay differences, as the Berichrom assay used in that study [48] typically yields higher activity values [43]. Our findings align more with another study [49] using Sta-Stachrom, which also reported increased recurrence risk below 70%. However, values between 70 and 80% may still be clinically relevant, especially for patients with ATE, since more than half of our ATE cases fell within this range. Given the variability in assay performance, ATD characterization might provide a more reliable and clinically meaningful approach to risk stratification.

Our study has some limitations. As a single-center study of a rare condition, the small sample size limited statistical power, restricted comparisons among ATD subtypes and variants, and precluded adjustment for covariates. Adjusting for additional thrombophilia would likely not affect our conclusions, as most patients with thrombophilia carried a type IIHBS variant, which would only reinforce the observed lower VTE risk. Moreover, data on the type and duration of anticoagulant or antiplatelet therapy after the first event and other ATE risk factors were largely unavailable, potentially affecting recurrence assessment. Given the observed differences among variants, genotype-phenotype correlations may be biased, as most type II (IIHBS) patients carried the AT Padua I variant. Adaptations of some assay protocols may have affected diagnostic performance. Nevertheless, the main findings are unlikely to change, as Sta-Stachrom, one of the two most sensitive assays, used an adapted protocol, while the automated assay with the lowest sensitivity (Hemosil) remained fully IVDR-compliant.

5. Conclusion

Our findings highlight that diagnostic sensitivity varies significantly across commercial AT activity assays and cannot be predicted based on the enzyme used in the assay or the ATD subtype. Nevertheless, Innovance and Sta-Stachrom, demonstrate high sensitivity and are promising for reliable ATD detection, while others appear less suitable, at least in populations like ours. Functional assays can identify patients at increased thrombotic risk even without a detectable genetic cause, underscoring the value of sensitive assay as a first-line diagnostic test. However, we endorse the suggestion [31] to routinely complement functional assays with targeted genetic testing in patients with normal AT activity but suspected ATD, to detect common variants missed due to transient deficiency (e.g., AT Dublin) or unique molecular traits (e.g., AT Cambridge II). To optimize patient management, it is essential to consider the heterogeneous clinical phenotype of ATD. Since AT activity does not fully reflect this heterogeneity, the ATD (sub)type should be determined through AT biochemical methods when genetic testing is unavailable. Although type IIHBS presents a lower risk for VTE, its diagnosis remains important, because certain variants, such as AT Basel, are associated with early-onset ATE, supporting the need for ATD screening in young patients with ATE.

CRediT authorship contribution statement

Tamara Rojnik: Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Robert Šket:** Writing – review & editing, Validation, Software. **Barbara Slapnik:** Writing – review & editing, Validation. **Blaž Vrhovšek:** Writing – review & editing, Software. **Alenka Mavri:** Writing – review & editing, Resources. **Maruša Debeljak:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Mojca Božič Mijovski:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Ethics approval and consent to participate

The protocol was approved by the Slovenian National Medical Ethics Committee (0120–374/2020/3).

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Declaration of competing interest

At the Laboratory for Hemostasis and Atherothrombosis, University Medical Centre Ljubljana, AT activity is measured using the Innovance assay on Sysmex® CS-2500 and CS-5100 analyzers (Siemens Healthineers). All authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2026.109689.

Data availability

The data used in this study are available from the corresponding author on reasonable request.

References

[1] G.W. Moore, Thrombophilia screening: not so straightforward, Semin. Thromb. Hemost. 50 (8) (2024) 1131–1152, https://doi.org/10.1055/s-0044-1786807.
[2] A.B. Alnor, C. Gils, P.J. Vinholt, Venous thromboembolism risk in adults with hereditary thrombophilia: a systematic review and meta-analysis, Ann. Hematol. 103 (10) (2024) 4285–4294, https://doi.org/10.1007/s00277-024-05926-2.
[3] C. Bravo-Perez, M.E. de la Morena-Barrio, V. Vicente, J. Corral, Antithrombin deficiency as a still underdiagnosed thrombophilia: a primer for internists, Pol. Arch. Intern. Med. 130 (10) (2020) 868–877, https://doi.org/10.20452/pamw.15371.

[4] M. Kruijt, C.M. Cobbaert, L.R. Ruhaak, Antithrombin: deficiency, diversity, and the future of diagnostics, Mass Spectrom. Rev. (2025), https://doi.org/10.1002/mas.21929.
[5] J. Corral, M.E. de la Morena-Barrio, V. Vicente, The genetics of antithrombin, Thromb. Res. 169 (2018) 23–29, https://doi.org/10.1016/j.thromres.2018.07.008.
[6] The Human Gene Mutation Database (HGMD). https://www.hgmd.cf.ac.uk/ac/index.php, 2025 (accessed 5 November 2025).
[7] E.M. Van Cott, C. Orlando, G.W. Moore, et al., Recommendations for clinical laboratory testing for antithrombin deficiency; communication from the SSC of the ISTH, J. Thromb. Haemost. 18 (1) (2020) 17–22, https://doi.org/10.1111/jth.14648.
[8] R. Gindele, Z. Olah, P. Ilonczai, et al., Founder effect is responsible for the p.Leu131Phe heparin-binding-site antithrombin mutation common in Hungary: phenotype analysis in a large cohort, J. Thromb. Haemost. 14 (4) (2016) 704–715, https://doi.org/10.1111/jth.13252.
[9] J. Corral, D. Hernandez-Espinosa, J.M. Soria, et al., Antithrombin Cambridge II (A384S): an underestimated genetic risk factor for venous thrombosis, Blood 109 (10) (2007) 4258–4263, https://doi.org/10.1182/blood-2006-08-040774.
[10] M. Puurunen, P. Salo, S. Engelbarth, K. Javela, M. Perola, Type II antithrombin deficiency caused by a founder mutation Pro73Leu in the Finnish population: clinical picture, J. Thromb. Haemost. 11 (10) (2013) 1844–1849, https://doi.org/10.1111/jth.12364.
[11] C. Orlando, B. de la Morena-Barrio, I. Pareyn, et al., Antithrombin p.Thr147Ala: the first founder mutation in people of African origin responsible for inherited Antithrombin deficiency, Thromb. Haemost. 121 (2) (2021) 182–191, https://doi.org/10.1055/s-0040-1716531.
[12] P.C. Cooper, F. Coath, M.E. Daly, M. Makris, The phenotypic and genetic assessment of antithrombin deficiency, Int. J. Lab. Hematol. 33 (3) (2011) 227–237, https://doi.org/10.1111/j.1751-553X.2011.01307.x.
[13] J.S. Ungerstedt, S. Schulman, N. Egberg, J. Antovic, N. Blomback, Discrepancy between antithrombin activity methods revealed in Antithrombin Stockholm: do factor Xa-based methods overestimate antithrombin activity in some patients? Blood 99 (6) (2002) 2271–2272, https://doi.org/10.1182/blood-2001-11-0047.
[14] C. Orlando, C. Dreze, A. Evenepoel, S. Seneca, K. Jochmans, Diagnostic performance of commercial antithrombin activity assays: do we get what we expect? Thromb. Haemost. (2025) https://doi.org/10.1055/a-2628-4046.
[15] B. Kovacs, Z. Bereczky, Z. Olah, et al., The superiority of anti-FXa assay over anti-FIIa assay in detecting heparin-binding site antithrombin deficiency, Am. J. Clin. Pathol. 140 (5) (2013) 675–679, https://doi.org/10.1309/AJCPVY4Z9XZMFOTH.
[16] C. Orlando, O. Heylen, W. Lissens, K. Jochmans, Antithrombin heparin binding site deficiency: a challenging diagnosis of a not so benign thrombophilia, Thromb. Res. 135 (6) (2015) 1179–1185, https://doi.org/10.1016/j.thromres.2015.03.013.
[17] R. Gindele, A. Selmeczi, Z. Olah, et al., Clinical and laboratory characteristics of antithrombin deficiencies: a large cohort study from a single diagnostic center, Thromb. Res. 160 (2017) 119–128, https://doi.org/10.1016/j.thromres.2017.10.023.
[18] S. Feddersen, M. Nybo, Discordant diagnoses obtained by different approaches in antithrombin mutation analysis, Clin. Biochem. 47 (13–14) (2014) 1337–1340, https://doi.org/10.1016/j.clinbiochem.2014.06.013.
[19] J. Corral, C. Bravo-Perez, M.E. de la Morena-Barrio, Hidden antithrombin defects: time to change and improve our thrombophilia diagnostic approach, J. Thromb. Haemost. 23 (8) (2025) 2399–2402, https://doi.org/10.1016/j.jtha.2025.05.021.
[20] W. Zeng, L. Tang, X.R. Jian, et al., Genetic analysis should be included in clinical practice when screening for antithrombin deficiency, Thromb. Haemost. 113 (2) (2015) 262–271, https://doi.org/10.1160/TH14-05-0446.
[21] S.V. Konstantinides, G. Meyer, C. Becattini, et al., 2019 ESC guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the European Respiratory Society (ERS), Eur. Heart J. 41 (4) (2020) 543–603, https://doi.org/10.1093/eurheartj/ehz405.
[22] Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU (Text with EEA relevance). Document 02017R0746–20250110. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02017R0746-20250110, 2025.
[23] S. Richards, N. Aziz, S. Bale, et al., Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology, Genet. Med. 17 (5) (2015) 405–424, https://doi.org/10.1038/gim.2015.30.
[24] T. Pascreau, C. Auditeau, D. Borgel, Hemostatic defects in congenital disorders of glycosylation, Res. Pract. Thromb. Haemost. 7 (3) (2023) 100142, https://doi.org/10.1016/j.rpth.2023.100142.
[25] D. Provaznikova, M. Matyskova, I. Capova, et al., Seventeen novel SERPINC1 variants causing hereditary antithrombin deficiency in a Czech population, Thromb. Res. 189 (2020) 39–41, https://doi.org/10.1016/j.thromres.2020.02.025.
[26] M. Alhenc-Gelas, G. Plu-Bureau, J. Hugon-Rodin, V. Picard, M.H. Horellou, GFHT study group on genetic thrombophilia, thrombotic risk according to SERPINC1 genotype in a large cohort of subjects with antithrombin inherited deficiency, Thromb. Haemost. 117 (6) (2017) 1040–1051, https://doi.org/10.1160/TH16-08-0635.
[27] E. Wypasek, J. Corral, M. Alhenc-Gelas, et al., Genetic characterization of antithrombin, protein C, and protein S deficiencies in polish patients, Pol. Arch. Intern. Med. 127 (7–8) (2017) 512–523, https://doi.org/10.20452/pamw.4045.
[28] B. Luxembourg, D. Delev, C. Geisen, et al., Molecular basis of antithrombin deficiency, Thromb. Haemost. 105 (4) (2011) 635–646, https://doi.org/10.1160/TH10-08-0538.
[29] A.D. Kjaergaard, O.H. Larsen, A.M. Hvas, P.H. Nissen, SERPINC1 variants causing hereditary antithrombin deficiency in a Danish population, Thromb. Res. 175 (2019) 68–75, https://doi.org/10.1016/j.thromres.2019.01.022.
[30] B. Luxembourg, A. Pavlova, C. Geisen, et al., Impact of the type of SERPINC1 mutation and subtype of antithrombin deficiency on the thrombotic phenotype in hereditary antithrombin deficiency, Thromb. Haemost. 111 (2) (2014) 249–257, https://doi.org/10.1160/TH13-05-0402.
[31] C. Bravo-Perez, M.E. de la Morena-Barrio, B. de la Morena-Barrio, et al., Molecular and clinical characterization of transient antithrombin deficiency: a new concept in congenital thrombophilia, Am. J. Hematol. 97 (2) (2022) 216–225, https://doi.org/10.1002/ajh.26413.
[32] M. Wojcik, M.E. de la Morena-Barrio, J. Michalik, et al., A series of 10 polish patients with thromboembolic events and antithrombin deficiency: two new c.1154-1 G>C and c.1219-534 a>G SERPINC1 gene splicing mutations, Blood Coagul. Fibrinolysis 30 (5) (2019) 193–198, https://doi.org/10.1097/MBC.0000000000000816.
[33] M. Caspers, A. Pavlova, J. Driesen, et al., Deficiencies of antithrombin, protein C and protein S - practical experience in genetic analysis of a large patient cohort, Thromb. Haemost. 108 (2) (2012) 247–257, https://doi.org/10.1160/TH11-12-0875.
[34] M.E. de la Morena-Barrio, I. Martinez-Martinez, C. de Cos, et al., Hypoglycosylation is a common finding in antithrombin deficiency in the absence of a SERPINC1 gene defect, J. Thromb. Haemost. 14 (8) (2016) 1549–1560, https://doi.org/10.1111/jth.13372.
[35] The Genome Aggregation Database (gnomAD). https://gnomad.broadinstitute.org/, 2025 (accessed 5 November 2025).
[36] G. Castaldo, A.M. Cerbone, A. Guida, et al., Molecular analysis and genotype-phenotype correlation in patients with antithrombin deficiency from southern Italy, Thromb. Haemost. 107 (4) (2012) 673–680, https://doi.org/10.1160/TH11-09-0671.
[37] B. de la Morena-Barrio, C. Orlando, A. Sanchis-Juan, et al., Molecular dissection of structural variations involved in Antithrombin deficiency, J. Mol. Diagn. 24 (5) (2022) 462–475, https://doi.org/10.1016/j.jmoldx.2022.01.009.
[38] R. Cifuentes, J. Padilla, M.E. de la Morena-Barrio, et al., Usefulness and limitations of multiple ligation-dependent probe amplification in Antithrombin deficiency, Int. J. Mol. Sci. 24 (5) (2023), https://doi.org/10.3390/ijms24055023.
[39] E. Danese, M. Montagnana, M. Gelati, G. Lippi, The role of epigenetics in the regulation of hemostatic balance, Semin. Thromb. Hemost. 47 (1) (2021) 53–62, https://doi.org/10.1055/s-0040-1718400.
[40] M. Olsson Lindvall, A. Angerfors, B. Andersson, et al., Comparison of DNA methylation profiles of hemostatic genes between liver tissue and peripheral blood within individuals, Thromb. Haemost. 121 (5) (2021) 573–583, https://doi.org/10.1055/s-0040-1720980.
[41] K. Javela, S. Engelbarth, L. Hiltunen, P. Mustonen, M. Puurunen, Great discrepancy in antithrombin activity measured using five commercially available functional assays, Thromb. Res. 132 (1) (2013) 132–137, https://doi.org/10.1016/j.thromres.2013.05.012.
[42] R. Gindele, K. Penzes-Daku, G. Balogh, et al., Investigation of the differences in Antithrombin to heparin binding among Antithrombin Budapest 3, Basel, and Padua mutations by biochemical and in silico methods, Biomolecules 11 (4) (2021), https://doi.org/10.3390/biom11040544.
[43] T. Rojnik, N. Sedlar, N. Turk, A. Kastrin, M. Debeljak, M. Bozic Mijovski, Comparison of antithrombin activity assays in detection of patients with heparin binding site antithrombin deficiency: systematic review and meta-analysis, Sci. Rep. 13 (1) (2023) 16734, https://doi.org/10.1038/s41598-023-43941-x.
[44] J. Navarro-Fernandez, M.E. de la Morena-Barrio, J. Padilla, et al., Antithrombin Dublin (p.Val30Glu): a relatively common variant with moderate thrombosis risk of causing transient antithrombin deficiency, Thromb. Haemost. 116 (1) (2016) 146–154, https://doi.org/10.1160/TH15-11-0871.
[45] V. Roldan, A. Ordonez, F. Marin, et al., Antithrombin Cambridge II (A384S) supports a role for antithrombin deficiency in arterial thrombosis, Thromb. Haemost. 101 (3) (2009) 483–486, https://doi.org/10.1160/TH08-09-0583.
[46] T. Chiasakul, E. De Jesus, J. Tong, et al., Inherited thrombophilia and the risk of arterial ischemic stroke: a systematic review and Meta-analysis, J. Am. Heart Assoc. 8 (19) (2019) e012877, https://doi.org/10.1161/JAHA.119.012877.
[47] G. Kenet, L.K. Lutkhoff, M. Albisetti, et al., Impact of thrombophilia on risk of arterial ischemic stroke or cerebral sinovenous thrombosis in neonates and children: a systematic review and meta-analysis of observational studies, Circulation 121 (16) (2010) 1838–1847, https://doi.org/10.1161/CIRCULATIONAHA.109.913673.
[48] M.N. Di Minno, F. Dentali, R. Lupoli, W. Ageno, Mild antithrombin deficiency and risk of recurrent venous thromboembolism: a prospective cohort study, Circulation 129 (4) (2014) 497–503, https://doi.org/10.1161/CIRCULATIONAHA.113.003756.
[49] J. Sokol, J.F. Timp, S. le Cessie, et al., Mild antithrombin deficiency and risk of recurrent venous thromboembolism: results from the MEGA follow-up study, J. Thromb. Haemost. 16 (4) (2018) 680–688, https://doi.org/10.1111/jth.13960.