Received: 2 March 2023 | Revised: 14 July 2023 | Accepted: 18 July 2023

https://doi.org/10.1016/j.rpth.2023.102197

REVIEW ARTICLE

Thromboembolic risk with gender-affirming hormone therapy: potential role of global coagulation and fibrinolysis assays

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Handling Editor: Dr Suzanne Cannegieter.

Abstract

Gender-affirming hormonal therapies are a critical component of the care of transgender individuals. Transgender people are commonly prescribed estrogen or testosterone to promote male-to-female or female-to-male transitions and to preserve gender-specific characteristics long-term. However, some exogenous hormones, especially certain estrogen preparations, are an established risk factor of thrombosis. As the number of individuals seeking gender-based care is rising, there is an urgent need to identify and characterize the mechanisms underlying hormone-associated thrombosis and incorporate this information into clinical algorithms for diagnosis and management. Herein, we discuss historical evidence on the incidence of thrombosis and changes in plasma composition in transgender and cisgender cohorts. We present 3 case studies to demonstrate knowledge gaps in thrombosis risk stratification and prediction tools. We also present data from *in vitro* coagulation and fibrinolysis assays and discuss how information from these kinds of assays may be used to help guide the clinical management of transgender individuals.

KEYWORDS
estrogens, fibrinolysis, risk factors, thrombosis, transgender

Essentials

- Some exogenous estrogens used for gender-affirming care are associated with thrombotic risk.
- Current literature is discussed and expanded with 3 case studies.
- Research-based global coagulation assays illuminate potential hypercoagulability.
- Collaborations with basic researchers could improve transgender health care.

1 | EPIDEMIOLOGIC AND CLINICAL DATA ON TRANSGENDER INDIVIDUALS

Data from epidemiologic, individual, and population studies implicate hormones as a driver of thrombotic risk.

1.1 | Transgender epidemiology

Worldwide, over 25 million individuals identify as transgender [1]. An estimated 6.7 to 7.7 assigned-male-at-birth individuals and 2.6 to 3.3 assigned-female-at-birth individuals per 100,000 people have transgender identity [2,3]. In the United States, the estimated prevalence of transgender individuals has doubled since 2011 to 1.4 million individuals [4]. Both child and adolescent referrals to gender clinics have increased, potentially reflecting the gradual depathologization and destigmatization of transgenderism, increased support from parents to enable gender transitions, and/or increased access to transgender health care programs [5]. Across Europe and Southeast Asia, the approximate 3:1 ratio of assigned-male-at-birth:assigned-female-at-birth individuals presenting as transgender is consistent (among individuals of all ages), suggesting the influence of culture on transgender identity is relatively small [3]. In *adolescent* cohorts of transgender individuals, assigned-female-at-birth persons are more common [5,6]. However, *adults* newly presenting with transgender identity are far more commonly assigned-male-at-birth. The prevalence ratio of male-to-female (MTF) to female-to-male (FTM) transgender individuals in the general population is 3:1 [5,7]. MTF individuals have increased mortality compared with age-matched *cis* subjects, whereas FTM individuals have similar mortality as age-matched *cis* subjects [7]. Since data linking testosterone-based gender-affirming hormone therapy (GAHT) for FTM individuals to heightened thrombosis risk are weaker and controversial, we focus herein on MTF individuals [8–13]. To care appropriately for these patients, further understanding of mechanisms leading to GAHT-associated thrombosis, as well as better ability to detect thrombotic risk, are needed.

1.2 | Case reports of thrombosis in patients on hormonal therapy

Thromboembolic events were documented as early as the 1970s in MTF-transitioning individuals receiving GAHT. Most studies have reported venous thrombosis or thromboembolism, including deep vein thrombosis with or without pulmonary embolism [14–17], cerebral venous thrombosis [18–20], and retinal vein occlusion [21]. MTF patients have also presented with arterial thrombosis, including myocardial infarction and ischemic stroke [22,23]. Overall, patients had 2-to-40-year-long (and variable) histories of GAHT, including estrogens (mestranol, ethinyl estradiol, conjugated estrogens) and progestogens (progesterone, levonorgestrel, cyproterone acetate). In many cases, other risk factors for venous thromboembolism and/or cardiovascular disease were present, including age >50 [17,19,20], prothrombin G20210A mutation [15], long-haul travel [16], obesity, and smoking [21]. Together, these observations reveal considerable heterogeneity in both risk factors and clinical presentation in individuals presenting with GAHT-associated thrombosis.

1.3 | Population studies of thrombosis in MTF transgender patients

Epidemiologic studies have confirmed the anecdotal reports of thrombosis in MTF transgender individuals using GAHT. Retrospective cohort studies from the Netherlands associated a daily regimen of 100-μg ethinyl estradiol and 100-mg cyproterone acetate (which is unavailable in the United States) with a 20-to-45-fold increase in venous thromboembolic risk compared with the general male population [24,25]. In a cohort of 2,236 MTF transgender patients, Gooren et al. [26] found that oral ethinyl estradiol is associated with a 6% to 8% incidence of venous thromboembolism. In a cohort of Belgian *trans* women receiving heterogeneous estrogen formulations, 5% to 6% of patients developed venous thromboembolism during GAHT use (mean, 7.4 years; range, 2.5-32.2 years), and this typically occurred within the first year of GAHT use [27,28]. Maraka et al. [29] performed a meta-analysis of 29 relevant studies and similarly identified a 5% rate of thrombosis in MTF cohorts, in contrast to a 0.1% rate in FTM cohorts. More recent studies from the United States showed 5.5% venous thromboembolism rates associated with GAHT in a cohort of nearly 3,000 MTF patients over an 8-year observation period [9]. In contrast to the Belgian cohorts, the United States cohort's hazard ratios were significantly greater than that of *cis* men and women at 8-year follow-up (16.7 and 13.7, respectively) compared with 2-year follow-up (4.1 and 3.4, respectively), suggesting an inflection point of increased venous thromboembolism risk around 2 years of follow-up [9]. A study from the Netherlands found that *trans* women using GAHT have a significantly increased standardized incidence ratio of venous thromboembolism compared with both *cis* men and *cis* women (4.55 and 5.52, respectively) across 22,830 total years of follow-up [13]. Notably however, several of these studies did not control for co-existing risk factors that may inflate risk (eg, smoking), which may account for differences in cumulative risk estimates.

Nonetheless, sufficient evidence supports the association between ethinyl estradiol and conjugated equine estrogen use and venous thromboembolic risk in postmenopausal women [30] and transgender women, that the latest edition of the Standard of Care from the World Professional Association for Transgender Health (WPATH) recommends avoiding ethinyl estradiol and conjugated equine estrogens in transgender patients [31]. WPATH suggests health care professionals prescribe transdermal estrogen for transgender people who are at higher risk of developing venous thromboembolism, based on age >45 years or a previous history of venous thromboembolism [31]. Estrogen-sparing interventions like antiandrogen therapy are also considered for individuals with indications of higher thrombosis risk [31]. Although these studies have resulted in Society recommendations to mitigate thromboembolic risk

in transgender women, the underlying mechanisms promoting thrombosis in this cohort remain unknown.

2 | FUNCTIONAL IMPACT OF SEX HORMONE THERAPY ON COAGULATION PROTEINS

Both sex hormones and hormone therapy produce changes in plasma composition and coagulation potential.

2.1 | Plasma changes following sex hormone initiation

Prothrombotic effects of exogenous hormone administration have been extensively documented in cisgender cohorts, and information from these studies may be extrapolated at least to some extent to transgender women. Young *cis* women taking oral contraceptives or postmenopausal *cis* women taking hormone replacement therapy experience a 2-to-5-fold increased risk of thrombosis [32–37]. Changes observed in plasma of these women suggest a hypercoagulable shift in plasma composition: increased levels of procoagulant factors (fibrinogen, prothrombin, and factors (F)VII and FVIII) and decreased levels of anticoagulant factors (antithrombin, protein S) [38–47]. In these cohorts, estrogen dose positively correlated with thromboembolic risk. *Trans* women may be administered 2-to-15 times the dose that *cis* women receive because high doses are needed for feminization [48–50].

Increased levels of procoagulant proteins have also been detected in plasma from transgender women using exogenous estrogens, including FVII, FIX, FXI, and von Willebrand factor [51–53]. Reduced anticoagulant proteins have also been observed, including protein C, protein S, and antithrombin [11,22,52]. Another study reported decreased fibrinolytic pathway proteins, including tissue plasminogen activator (tPA), urokinase plasminogen activator, and plasminogen activator inhibitor type 1 (PAI-1) [53]. Increased inflammatory cytokines such as interleukin (IL)-1, IL-6, and IL-8 have also been detected in these individuals [51]. The magnitude of these changes is influenced by age, and depends on the route of hormone administration; oral estrogen produces greater changes compared with transdermal administration [51,52].

In sum, these studies suggest that a systemic, procoagulant shift in plasma composition in *trans* women taking estrogen may predispose them to thrombosis. However, the cumulative effect of these multifaceted changes is difficult to discern based on quantitative levels of each individual protein. Standard clinical clotting assays (eg, activated partial thromboplastin time, prothrombin time) are exquisitely sensitive to *hypo*-coagulability, but generally lack sensitivity to *hyper*-coagulability. Therefore, available clinical measurements are ill-informed to predict thrombosis. Development and use of robust methods for tracking procoagulant profiles of transgender women is imperative to facilitate the risk/benefit analysis between thrombosis and the quality-of-life benefit gained through GAHT [31].

2.2 | Use of global coagulation assays to measure blood and plasma function

Whole blood or plasma-based coagulation assays (so-called "global assays") are used to measure the dynamics of blood clot formation, or specific kinetics of thrombin generation, fibrin polymerization, and/or fibrinolysis. When compared with samples from healthy control individuals, these tests can reveal relative (im)balances in procoagulant, anticoagulant, and fibrinolytic activity stemming from changes in one or several blood components. Established whole blood-based assays (eg, thromboelastography or rotational thromboelastometry) can evaluate coagulation and fibrinolytic potential in the presence of all whole blood components—soluble coagulation factors, platelets, red blood cells, and leukocytes—but require fresh samples [54]. Plasma-based assays (eg, thrombin generation assays including calibrated automated thrombography, and turbidimetry) are performed in the absence of cells, but offer increased flexibility and a collaborative advantage since they can be performed on frozen, thawed samples [55]. For each of these assays, reactions are triggered by the addition of a coagulation initiator, typically tissue factor (extrinsic pathway) or kaolin (intrinsic pathway). Subsequent events (thrombin generation, fibrin formation, and/or fibrinolysis) are monitored continuously over time. Analysis of the reaction kinetics reveals parameters that can be used to describe the sequence of events leading to clot formation and/or dissolution.

Importantly, these global assays can be manipulated to mimic vascular contributions to coagulation and fibrinolysis and increase the sensitivity to specific pathways. For example, *in vivo*, thrombomodulin expressed by endothelial cells mediates activation of protein C by thrombin. Activated protein C (APC) is an important negative feedback molecule that degrades procoagulant cofactors (FVa, FVIIIa) and down-regulates thrombin generation. The effects of thrombomodulin and APC activity can be observed *in vitro* by the addition of either thrombomodulin or APC to these reactions [56]. In this way, the prothrombotic state associated with insensitivity to the APC pathway ("APC resistance") can be detected. This mechanism may be particularly important in the setting of hormone-associated thrombosis. For example, postmenopausal *cis* women who use hormone replacement therapy develop APC resistance, which has been attributed to decreased levels of tissue factor pathway inhibitor and protein S [57–60]. APC resistance is also noted during normal pregnancy, possibly influenced by increased FVIII and von Willebrand factor in addition to decreased protein S [61]. Similar changes and APC resistance have been noted in oral contraceptive users [62–64]. Reviews detailing APC resistance and hormone-associated thrombosis in cisgender women are available [65–68].

Despite the potential for global assays to reveal functional abnormalities associated with hormone use, only a few studies have used these methods to identify hypercoagulability in transgender individuals. Both thromboelastography and thrombin generation assays detected elevated procoagulant activity in plasma from *trans* women using GAHT vs cisgender men [69]. Further, functional APC resistance was observed in thrombin generation assays after GAHT and

attributed to decreased protein S antigen [11]. Turbidimetric fibrinolysis assays indicated enhanced fibrinolytic potential in *trans* women [53,69]. Collectively, these observations suggest *in vitro* global assays may be helpful for identifying hypercoagulability including APC resistance in transgender women using GAHT, and used to evaluate their thrombotic risk and guide decision-making.

3 | INTEGRATION OF CASE-BASED EVIDENCE WITH ROUTINE AND GLOBAL COAGULATION ASSAYS

To illustrate the clinical complexity of transgender care and illuminate how information from global assays may be incorporated into treatment plans for transgender women seeking GAHT, we present 3 clinical case studies. All studies were conducted in accordance with the principles of the Declaration of Helsinki. All participants were adults and provided written informed consent. The protocol, amendments, and informed consent forms were approved by the Institutional Review Board of University Hospitals Cleveland Medical Center (Case 12Z05, IRB # 09-90-195) and the Louis Stokes Cleveland Veterans Administration Medical Center (CY18-051).

A transgender health care provider with extensive experience in prescribing GAHT identified these patients as being at high thrombotic risk related to estrogen therapy. This provider consulted with hematology to identify strategies for decreasing the patients' risk. Clinical testing, performed at the time of intake, included standard coagulation panels and thrombophilia testing per physician recommendation. Treatment decisions for each of these patients were made in real-time. Plasma was also obtained for subsequent analysis using global coagulation and fibrinolysis assays. Three global assays were employed to compare transgender coagulation profiles to those of a reference cohort of age-matched individuals (6 *cis* men and 5 *cis* women age-matched to the transgender individuals, Table 1). Thrombin generation potential was observed by calibrated automated thrombography, and fibrin formation and fibrinolytic potential were evaluated by turbidimetry, as described previously [56,70]. The treatment decisions that had been made were then reviewed in the context of these data. A summary of these discussions is presented for each case. These case studies are meant to facilitate discussion of these nuanced clinical situations and are not intended to represent the broad transgender population.

TABLE 1 Demographic and clinical characteristics of reference cohort.

Demographic or clinical characteristic	*Cis* Women *n* = 5	*Cis* Men *n* = 6
Age (y), mean ± SD	53.2 ± 14.3	47.2 ± 15.2
Race – *N* (%)		
White	4 (80%)	4 (67%)
African American	1 (20%)	2 (33%)
Other	0%	0%
Body mass index – kg/m^2, mean ± SD	26 ± 7.1	26.84 ± 5.5
Estimated Glomerular filtration rate – *N* (%)		
≥80 mL/min	4 (80%)	4 (30%)
50-80 mL/min	1 (20%)	2 (70%)
30-50 mL/min	0	0
Presence of sickle cell trait among tested individuals[a]	0	0
Diabetes Mellitus Type II – *N* (%)	2 (40%)	1 (17%)
Hemoglobin A1C – mean %	7.3	7.5
Comorbidities – incidence (%)		
Hypertension	20%	50%
Hyperlipidemia	40%	50%
Coronary artery disease[b]	0%	0%
Arterial thrombotic events[c]	0%	0%
Venous thromboembolic events[d]	0%	0%

[a]Hemoglobin electrophoresis results were available for 1 *cis* woman and 2 *cis* men.
[b]Coronary artery disease: documented in patients' medical record and identified by prior acute myocardial infarction, positive stress test, evidence of coronary stenosis by cardiac catheterization, or presence of cardiac stents.
[c]Arterial thrombotic events: defined as *de novo* or embolic thrombosis in an artery.
[d]Venous thromboembolic events: defined as *in situ* or embolic thrombosis in a deep vein. Superficial vein thrombosis was excluded from this group.

3.1 | Clinical question #1: can laboratory testing inform a discussion about the risks of venous thromboembolism with GAHT in transgender women with a family history of thrombosis?

3.1.1 | Presentation

A *trans* White woman in her 40s with a history of gender-affirmation surgery presented to the primary care clinic. Her medical history was notable for hypertension, hyperlipidemia, and post-traumatic stress disorder. She had been on estrogen therapy (oral estradiol 2 mg daily) for 3 years with no previous thrombotic events or other adverse effects. The patient requested an escalation to high-dose estrogen therapy to promote gender transition. However, she had a family history of arterial thrombotic events wherein her sister had experienced recurrent cerebral vascular accidents since 14 years of age. Hematology was consulted for thrombotic risk assessment prior to estrogen dose escalation.

3.1.2 | Treatment strategy based on routine coagulation testing and clinical assessment

All measured values were within their respective normal ranges (Table 1), suggesting no overt risk factor for a thrombotic event. Genetic thrombophilia testing was considered but ultimately forgone since family history of arterial events is not commonly associated with genetic thrombophilia (eg, factor (F) V Leiden or prothrombin G20210A mutation) [71,72]. Antiphospholipid syndrome, hyperhomocysteinemia, and hyperlipidemia measures were obtained, and mixed hyperlipidemia was identified, indicating increased risk of arterial disease. There are no meaningful provisions in the WPATH guidelines for dose-escalation regimens in low-risk transgender women, defined as age <45 years with no personal history of thrombosis), who are already on estrogen therapy and seek to further increase their feminization treatment [72]. Therefore, since clinical testing did not reveal an underlying high-risk hypercoagulable state, we saw no contraindication in dose escalation of estrogen therapy [73]. In this case, 0.2 mL of 20 mg/mL estradiol (4 mg) with weekly injections was prescribed with the additional recommendation to optimize treatment for her hypertension and hyperlipidemia.

3.1.3 | Analysis using global plasma coagulation and fibrinolysis assays

Compared to the cisgender reference cohort (Table 1), plasma from this individual showed an enhanced velocity and peak of thrombin generation, and increased endogenous thrombin potential (area under the curve) (Figure A). When thrombomodulin was included in thrombin generation reactions to promote the APC anticoagulant pathway, we noted APC resistance with highly elevated thrombin generation velocity and peak compared with the reference cohort (Figure B). Fibrin formation appeared normal (Figure C); however, fibrinolysis (return to baseline turbidity) was slightly delayed (Figure D). Collectively, these data suggest this individual has detectable plasma hypercoagulability even at her low dose of GAHT.

3.1.4 | Alternative treatment approach based on global plasma coagulation assays

Enhanced thrombin generation and diminished fibrinolysis were detected in plasma from this individual, despite pharmacologic management of hypertension and hyperlipidemia, suggesting a

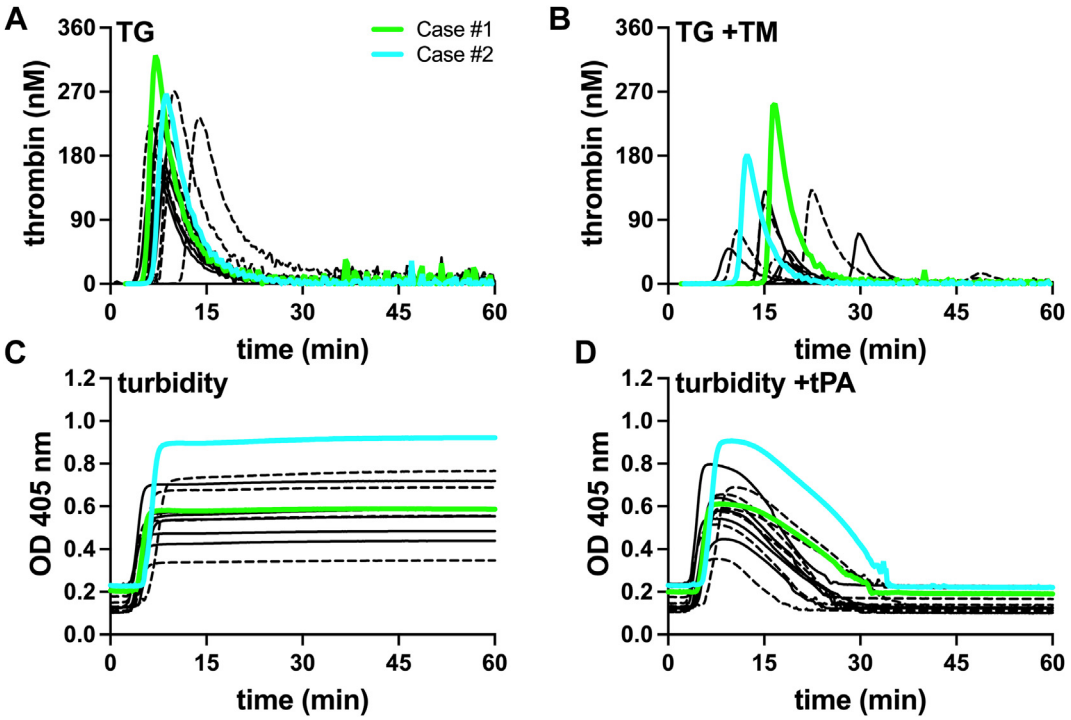

FIGURE Global plasma coagulation analyses. Citrated plasmas from cases #1 (green) and #2 (blue) and reference cisgender individuals (females, solid black; males, dashed black) were diluted 1:1. Thrombin generation was triggered with the addition of calcium (16.67 mM, final), phospholipids (4 μM, final), and tissue factor (Innovin diluted 1:15,000, final) in the **(A)** absence or **(B)** presence of rabbit thrombomodulin (5 nM, final). **(C)** Fibrin formation and **(D)** fibrinolysis were followed by turbidity at 405 nm triggered with calcium (10 mM, final), phospholipids (4 μM, final), and tissue factor (Innovin diluted 1:15,000, final) without and with tissue plasminogen activator ([tPA] 0.31 μg/mL, final), respectively. TG, thrombin generation; TM, thrombomodulin.

hypercoagulable state while receiving low-dose GAHT. These data may caution against further dose escalation of estrogen therapy. Had these data been available to our clinical team in real-time, we would have alternatively discussed more aggressive risk modification of hypertension and hyperlipidemia, and planned short-term follow-up to monitor progress before increasing estrogen dose.

3.2 | Clinical question #2: can laboratory testing guide risk stratification and treatment strategies in patients with an increased baseline risk for venous thromboembolism due to medical comorbidities?

3.2.1 | Presentation

A *trans* White woman in her 70s was seen in the clinic for chronic disease management. Her history was notable for hypertension, poorly controlled type 2 diabetes mellitus (HbA1c 8.6%), hyperlipidemia, and class 2 obesity (body mass index 38 kg/cm^2). She also had significant bilateral knee osteoarthritis and was generally sedentary. Although she had used GAHT (unknown formulation) for over 10 years, she self-discontinued it for approximately 15 months due to mental health issues including depression. More recently, she reported improvement in her mood and was interested in resuming GAHT. The patient had no personal or family history of coagulopathy; however, hematology was consulted for thrombotic risk stratification due to her significant comorbidities.

3.2.2 | Treatment strategy based on routine coagulation testing and clinical assessment

All clinically measured values were within their respective normal ranges (Table 2). Even with substantial prothrombotic comorbidities, no clear contraindications for GAHT were observed via available clinical diagnostics. Given her significant comorbidities, we cautiously recommended low-dose, transdermal estrogen. With low estrogen dose, transgender individuals commonly use concomitant antiandrogen therapy with compounds like spironolactone or cyproterone acetate (the latter is not approved in the United States) that inhibit testosterone synthesis [31,74,75]. Therefore, spironolactone was included in this patient's formulation. However, she developed chest pain in the interim and was admitted urgently for additional evaluation by Cardiology. Cardiac catheterization revealed significant coronary atherosclerosis but no acute myocardial infarction. The patient was discharged with the recommendation for aggressive modification of cardiovascular risk factors, including optimization of her diabetes, weight, and lipid profile. The WPATH guidelines recommend addressing modifiable risk factors, defined as smoking, obesity, and sedentary lifestyle, but do not provide insight on treatment options while these factors are being treated, which can take months or years to correct [72]. Faced with multiple chronic conditions, a poor social support system, and unstable housing, this individual continues to remain off GAHT but is closely followed by our multidisciplinary Transgender Clinic at the VA Medical Center Clinic where she has access to legal assistance, financial aid, home care, mental, and medical health.

3.2.3 | Analysis using global plasma coagulation and fibrinolysis assays

Compared to the reference cohort, this patient's thrombin generation appeared normal, although the peak and velocity were modestly enhanced compared with the mean of the reference group (Figure A). In the presence of thrombomodulin, the time parameters were shortened and the velocity and peak thrombin generation were enhanced, suggesting APC resistance (Figure B). Compared to the reference group, fibrin formation and fibrinolysis assays showed a substantially increased turbidity change (Figure C–D). Although increased turbidity change can be observed secondary to elevated plasma fibrinogen [76], this patient had normal fibrinogen levels (Table 2). Fibrinolysis assays also revealed a substantially delayed time to fibrinolysis (Figure D).

3.2.4 | Alternative treatment approach based on global plasma coagulation assays

The global plasma analyses indicated APC resistance and enhanced fibrin formation, which may serve as tangible evidence of hypercoagulability. In the clinical setting, this information may be used to provide patient education and facilitate shared decision-making discussions. Had these data been available in real-time, the clinical management of this patient would likely consist of an in-depth discussion with the patient regarding potential implications of her hypercoagulable profile. These data support the clinical decision to forego feminization therapy and estrogen exposure until her comorbidities were better managed.

3.3 | Clinical question #3: can laboratory studies guide GAHT management in patients on anticoagulation?

3.3.1 | Presentation

A *trans* White woman in her 70s presented to the emergency department with acute dyspnea. She was found to be in acute hypoxic respiratory failure which rapidly progressed to cardiac arrest, necessitating cardiopulmonary resuscitation. The patient had a medical history of chronic kidney disease stage III, hyperlipidemia, depression, gender dysphoria, and GAHT (oral estradiol 3 mg daily). A chest CT angiogram showed massive pulmonary embolism; therefore, the patient received tPA (50 mg bolus in the emergency department, then another 50 mg infusion over the next few hours) and was placed on

TABLE 2 Clinical measurements of transgender patients.

Laboratory test	Reference range	Patient #1	Patient #2	Patient #3
Prothrombin time	10-13.2 seconds		10.8	
Activated partial thromboplastin time	26-38 seconds		31	26
D-dimer	0-500 ng/mL fibrinogen equivalent units	385	394	33791→496→428
Fibrinogen	200-450 mg/dL	244	356	649→541
Fibrinogen antigen	180-350 mg/dL		409	
PAI-1 activity	0-27 International Units/mL	22	26	<4
PAI-1 antigen	4.0-43.0 ng/mL	37		
Alpha2-antiplasmin	80-150%	133	124	117
Antithrombin activity	75-135%	110		
Antithrombin antigen	72-124%	88		
Factor II G20210A mutation	Normal			Normal
Factor V Leiden mutation	Normal			Normal
Protein S, Free (LC)	57-157%			101
Protein S, total (LC)	60-150%			120
Protein S, functional (LC)	63-140%			108
Protein C	70-140 % activity			67→51
Anti-β2 glycoprotein 1 Ab IgM	0-32 β2 GP1 IgM units	<9→<9		<9
Anti-β2 glycoprotein 1 Ab IgG	0-20 β2 GP1 IgG units	<9→<9		<9
Anti-β2 glycoprotein 1 Ab IgA	0-25 β2 GP1 IgA units	<9→<9		<9
Anti-cardiolipin Ab IgM	0-10 phospholipid units	18→16		<10
Anti-cardiolipin Ab IgG	0-10 phospholipid units	<10→<10		<10
Anti-cardiolipin IgA	0-10 phospholipid units	<10→<10		<10
partial thromboplastin time (Lupus anticoagulant)	0-51.9 seconds	30.6		
dRVVT (LC)	0-47.0 seconds	45.1		
Lupus anticoagulant Interpretation (LC)	Not detected	Not detected		
Homocysteine	3.2-10.7 μmol/L	7.3	10	
Lipoprotein A	<=75.0 nmol/L	16.3	32.4	
Erythrocyte sedimentation rate	0-30 mm/hr	24→20		<1
C-reactive protein	0-9.99 mg/L	8.61→8.57		2.82
Total cholesterol	135-200 mg/dL	222	148	150
Low density lipoprotein	0-110 mg/dL	144	67	54
High density lipoprotein	35/80 mg/dL	35	34	37
Triglycerides	0-150 mg/dL	244	430	73
Hemoglobin A1c	3.6-5.7%	5.6	8.6	5.9

Arrows represent repeated measurements. Patient 2 underwent repeat diagnostics 3 months apart. For patient 3, D-dimer was measured at the time of pulmonary embolism, then 1 and 11 months later; fibrinogen levels were measured 24 hours apart in the week following the pulmonary embolism; Protein C was first measured a month after the pulmonary embolism, and then 10 months later.

Ab, antibody; dRVVT, dilute Russell viper venom test; LC, liquid chromatography; PAI-1, plasminogen activator inhibitor-1.

high-intensity i.v. heparin (18 units/kg/hour drip rate) anticoagulation. Upon hemodynamic stabilization, the patient received apixaban 10 mg oral twice daily for a total of 7 days including time on i.v. heparin, before she was transitioned to apixaban 5 mg oral twice daily and estradiol was discontinued. Three months later, the patient experienced worsening of her gender dysphoria and requested to resume hormonal therapy. Because of her previous thromboembolic event, hematology was consulted to assess thrombotic risk.

3.3.2 | Treatment strategy based on routine coagulation testing and clinical assessment

Genetic testing was sent for factor V Leiden and the prothrombin 20210 gene mutation, neither of which were present. Coagulation protein analysis revealed reduced PAI-1 and protein C (Table 2). PAI-1 inhibits tPA and subsequent plasmin generation; reduced PAI-1 activity may be expected to enhance fibrinolysis and protect against thrombosis. Conversely, reduced levels of the anticoagulant protein C could represent a procoagulant phenotype consistent with APC resistance.

Due to this patient's older age and history of thromboembolism, transdermal estradiol (0.025 mcg daily) was recommended. Compared to oral estrogen, transdermal estrogen carries lower thrombotic risk, but it is more difficult to achieve therapeutic plasma levels [11]. In addition, since transdermal formulations may not be as effective for treating gender dysphoria, progesterone was also prescribed. To safely provide GAHT while mitigating the known thromboembolic risk, this individual was advised to remain on long-term apixaban anticoagulation, per Society recommendations [31].

3.3.3 | Analysis using global plasma coagulation assays

Since this individual was receiving anticoagulation with apixaban at the time of blood collection, plasma coagulation activity would have been suppressed due to this factor Xa inhibitor and unlikely to show basal coagulation potential. Therefore, functional analysis of plasma from this individual was not performed, illustrating a limitation to the potential use of global coagulation and fibrinolysis assays in certain settings.

3.4 | Perspectives from the case studies

When individuals seeking GAHT also present with identifiable thrombotic risk factors, hematologists have limited clinical resources to detect blood hypercoagulability and integrate these measurements into a generalized risk assessment strategy. Data from global plasma-based assays may enhance conversations between clinicians and patients in developing a holistic GAHT plan. Together, these data and discussion show that global assays may be useful, but more robust studies are needed to fully understand their potential value in the context of GAHT management. In particular, these assays have relatively high inter-assay and inter-laboratory variability, are not standardized, and are not currently approved for diagnosing hypercoagulability even in cisgender individuals. Moreover, since coagulation assays show sex-specific effects, rationales for the appropriate reference population (eg, cisgender women or cisgender men) will require thoughtful consideration. Longitudinal measurements of transgender individuals before and after initiating GAHT may be needed to fully understand the effects of exogenous estrogens on plasma function and thrombotic risk.

4 | MULTIDISCIPLINARY CARE AND QUALITY OF LIFE

In addition to GAHT, transgender individuals may have additional acquired and/or genetic prothrombotic risk factors. For example, studies have identified a high prevalence of tobacco use and a disproportionate risk for poor cardiovascular outcomes among transgender individuals [77,78]. Moreover, risk factors may be differently present and have different impact on adolescents vs older individuals, further complicating risk assessment [79]. Managing GAHT in patients with sickle cell disease may be particularly challenging, yet there is little data to help guide exogenous hormone use in this population [80]. Discussions between physicians and patients should address modifiable risk factors, including smoking cessation, increasing physical activity, and managing comorbidities such as diabetes, dyslipidemia, and hypertension. Since there appears to be little or no risk of perioperative thrombosis in individuals after gender-affirmation therapy, there are no guidelines for managing estrogen therapy prior to, or immediately following, gender-affirmation surgery. However, hospitalization and surgery incur thrombotic risk, and decisions on hormone management and prophylactic anticoagulation are made in lieu of data, on a case-by-case basis [50,81–83].

Hematologists are increasingly likely to be consulted on cases such as those we describe and should be aware of—and sensitive to—the unique challenges transgender individuals face [84]. Appropriate GAHT is an important and multifaceted component of gender dysphoria care; beyond gender affirmation, GAHT improves mental health-related outcomes and quality-of-life scores among transgender people [85,86]. Adding to the nuanced risk-benefit analyses surrounding care in this population, transgender people have described a range of transphobic clinician behaviors, and some individuals fear being honest with clinicians [87–89]. Out of concerns about potential discrimination, many individuals rely on unsupervised self-administration of GAHT, increasing risk of complications, including thrombosis. Current guidelines recommend that surgeons who perform gender-affirming surgical procedures are trained with knowledge about diverse gender identities and expressions [31]. Nevertheless, approximately one-third of transgender people in the United States have negative encounters in health care settings leading to avoidance and delays in care [90]. Insufficient training, knowledge

gaps, limited scientific evidence and guidelines, gatekeeping practices, and stigmatizing beliefs hinder the ability to provide gender-affirming care. As such, a multidisciplinary team—primary care provider, endocrinologist, mental health provider, hematologist, case manager, social worker, and other personnel—should work together with transgender individuals seeking GAHT to optimize treatment [31,72]. As we have demonstrated, laboratory-based global coagulation assays may add a dimension to the qualitative and quantitative information available in the clinic. Thus, we advocate for close relationships between clinical hematologists being consulted for cases involving GAHT and basic scientists with access to dynamic coagulation assays. We propose that these collaborations will elevate care for these individuals and protect transgender lives.

5 | CONCLUSIONS

Given the risk of thrombosis associated with GAHT and increasing number of individuals seeking gender-affirming care worldwide, it is critical to understand risk factors and pathologic mechanisms leading thromboembolic complications in this population, as well as tools that may illuminate these phenotypes. GAHT should be individualized and part of a holistic approach to gender-affirming care. Hematology clinics need personalized risk assessment strategies to define risks for cardiovascular events, but are currently ill-equipped to predict thrombotic events. Basic scientists with expertise in global, dynamic coagulation assays may be the missing piece in the clinical management of transgender patients seeking GAHT.

ACKNOWLEDGMENTS

We thank the Veterans for their participation and the research staff for their help in carrying out this study.

FUNDING

This study was supported by funding from the National Institutes of Health (HL141791 and HL126974 [ASW], HL069768 [UNC/EGB], HL137695 [EXS]), Merit Review Award BX003851 (EXS), and the Oscar D. Ratnoff Endowed Professorship (EXS). The contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health, the United States Department of Veterans Affairs, or the United States Government. This article was also made possible by support from the Hemostasis and Thrombosis Research Society (HTRS), the leading North American professional society dedicated to research, mentoring, workforce development, and continuing medical education for physicians, investigators, and all health care professionals interested in advancing care for people with hemostatic and thrombotic disorders. At the core of this mission, HTRS supports the training of early-stage investigators pursuing careers in clinical, translational, or basic science research in hemostasis or thrombosis under the guidance of experienced mentors. These grants are meant to offer steppingstones toward the National Institutes of Health funding pipeline. As such, this project was mentored by two HTRS seasoned members (Evi X. Stavrou, MD and Alisa S. Wolberg, PhD) and features the research of two HTRS member trainees (Emma G. Bouck, PhD and Eduarda Grinsztejn, MD). *Research and Practice in Thrombosis and Haemostasis* is the leading journal affiliation of HTRS.

AUTHOR CONTRIBUTIONS

E.G.B. and E.G. wrote the first draft of the manuscript. All authors reviewed and edited the manuscript, and approved the final version of the manuscript.

RELATIONSHIP DISCLOSURE

The authors have no competing financial interests to declare.

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