Received: 2 November 2020 | Accepted: 26 January 2021

DOI: 10.1111/jth.15256

ORIGINAL ARTICLE

Effect of gender-affirming hormone use on coagulation profiles in transmen and transwomen

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Abstract

Background: The transgender population that uses gender-affirming hormone therapy (GAHT) is rapidly growing. The (side) effects of GAHT are largely unknown. We examined the effect of GAHT on coagulation parameters associated with venous thromboembolism (VTE) risk.

Methods: Factor (F)II, FIX, FXI, protein (p)C and free pS, fibrinogen, hematocrit, sex hormone-binding globulin, and normalized activated protein C ratio were measured in 98 transwomen (male sex at birth, female gender identity) and 100 transmen (female sex at birth, male gender identity) before and after 12 months of GAHT (oral or transdermal estradiol and anti-androgens in transwomen, transdermal or intramuscular testosterone in transmen). Mean paired differences in coagulation measurements were estimated with 95% confidence intervals (95% CI). Differences for route of administration and age were assessed with linear regression.

Results: After GAHT, transwomen had more procoagulant profiles with a mean increase in FIX: 9.6 IU/dL (95% CI 3.1–16.0) and FXI: 13.5 IU/dL (95% CI 9.5–17.5), and a decrease in pC: −7.7 IU/dL (95% CI −10.1 to −5.2). Changes in measures of coagulation were influenced by route of administration (oral vs. transdermal) and age. A higher sex-hormone binding globulin level after 12 months was associated with a lower activated protein C resistance. In transmen, changes were not procoagulant overall and were influenced by age. Differences for route of administration (transdermal vs. intramuscular) were small.

Conclusions: GAHT in transmen was not associated with apparent procoagulant changes, which provides some reassurance regarding VTE risk. In transwomen, GAHT resulted in procoagulant changes, which likely contributes to the observed increased VTE risk.

KEYWORDS
coagulation, estrogen, hormone therapy, testosterone, transgender persons

Manuscript handled by: Jean Connors

Final decision: Jean Connors, 26 January 2021

1 | INTRODUCTION

Both in health and disease there are important differences between women and men. This holds also true for the coagulation system, especially in light of its pathophysiological side, concerning hypercoagulability and its subsequent disease, venous thromboembolism (VTE). In VTE there are differences in risk factors, absolute incidence, presenting location, optimal management strategies, and prognosis between men and women.[1-4]

In women, hormones such as oral contraceptives and hormone therapy (HT) are important VTE risk factors.[5] Mechanistically, studies indicate that use of these hormones results in a procoagulant shift, with a consequential increased risk of VTE. Use of oral contraceptives (dependent on the dose of estrogen and type of progestogen) results in procoagulant changes with reported increases in factor (F) II, FVII, FVIII, FIX, FX, FXI, protein C, and fibrinogen; decreases in levels of antithrombin and protein S; and an increased resistance to activated protein C (APCr).[6-9] Similar procoagulant effects and subsequent increased VTE risk have been reported in women using estrogen containing HT for relief of menopause symptoms.[10-14]

Available data on hormone use in men, specifically testosterone, and the risk of VTE is less extensive. In a large cohort study, testosterone use in men was associated with an increased risk of VTE during the first 6 months of treatment (rate ratio:1.63, 95% confidence interval [CI] 1.12–2.37).[15] Studies on the effects of exogenous testosterone on the coagulation profile of men are inconclusive and there do not seem to be substantial procoagulant effects.[16,17] Moreover, testosterone is typically used in men who suffer from hypogonadism in which the underlying pathology may influence coagulation profiles.

A rapidly growing population in which estrogen or testosterone is frequently used concerns transgender persons who desire gender-affirming hormone therapy (GAHT). Transmen (female sex assigned at birth, male gender identity) typically receive testosterone, whereas transwomen (male sex assigned at birth, female gender identity) often receive estrogens (estradiol in particular), frequently combined with anti-androgens (often progestogenic cyproterone acetate).[18] Whether GAHT has similar procoagulant effects and subsequent risks is not yet extensively studied. Regarding the risk of VTE in the transgender population, some evidence is available.[19] Overall, transwomen using GAHT seem to be at an increased risk of VTE compared to cismen, for whom estimates of its incidence range from 41 to 167 per 10,000 person years in the literature, depending on the specific population studied.[20-22] Data on risk of VTE in transmen using GAHT are limited. In a recent large cohort study, the risk of VTE in transmen was not increased compared to women of the same age.[22] The effects of testosterone on the coagulation system in transmen, and estrogen with anti-androgens in transwomen is largely unknown. A previous study reported procoagulant changes in transwomen using estradiol with cyproterone acetate, and no procoagulant changes in transmen using testosterone; however, this study was limited by a small sample size.[23] In addition, the route of administration of GAHT may also influence the effect on the coagulation system. Previous studies found that both in women and transwomen (especially highly dosed) oral preparations induced more procoagulant changes and a higher VTE risk than transdermal preparations.[10,24-26]

Essentials

- The effects of gender-affirming hormone therapy (GAHT) on coagulation are largely unknown.
- Coagulation parameters were studied in 98 transwomen and 100 transmen 12 months after GAHT.
- Start of GAHT in transmen was not associated with apparent procoagulant changes.
- In transwomen GAHT resulted in procoagulant changes potentially associated with thrombotic risk.

In this study our aim was threefold. First, to assess whether GAHT has similar effects on the coagulation system as is observed in women using estrogens and men using testosterone, and whether these findings are in line with observations on VTE risk in the transgender population. Second, to investigate whether these changes are influenced by route of administration and age. Last, we explored whether changes in sex-hormone-binding globulin levels (SHBG), a previously described marker of procoagulant changes in women using oral contraceptives,[27] were similarly associated with changes in the coagulation profiles in this transgender population.

2 | METHODS

2.1 | Study design and subjects

This study is part of the European Network for the Investigation of Gender Incongruence (ENIGI), which is a collaboration among the gender identity clinics of Amsterdam (the Netherlands), Ghent (Belgium), Oslo (Norway), and Florence (Italy).[28] All participants in this study have been diagnosed with gender dysphoria by specialized psychologists. After the diagnostics, participants without a history of sex hormone use entered the ENIGI study if they provided informed consent. Exclusion criteria were the presence of a psychotic disorder, or not mastering the required language sufficiently at the site of participation. Based on the availability of blood samples, only participants who visited the gender clinic of the Amsterdam University Medical Center, Location Vrije Universiteit, the Netherlands have been included in the current study. This study was approved by the medical ethics committee of the Amsterdam University Medical Center, Location Vrije Universiteit in Amsterdam, the Netherlands.

For this study, blood was drawn twice. First at baseline, before participants commenced GAHT (estrogen and anti-androgens in transwomen and testosterone in transmen). The second sample was

obtained approximately 12 months after start of GAHT. All samples were obtained between August 2012 and September 2015. Blood was collected through venipuncture into vacutainers containing 0.105 M/0.109 M sodium citrate and subsequently centrifuged to obtain plasma samples. The plasma samples were stored at −80°C until analyses.

2.2 | Measures of coagulation

A total of eight measures of coagulation were investigated, which were selected based on their previously described effects in women (i.e., of estrogen use) and men (i.e.. of testosterone use).[17,29,30] These included levels of coagulation FII, FIX and FXI; fibrinogen and hematocrit; the natural anticoagulants protein S and protein C; and the APCr, which reflects a decreased sensitivity to the inhibition of APC on thrombin generation during coagulation.

FII, FIX, FXI, fibrinogen (Clauss method), protein C, and free protein S concentrations were determined using the HemosIL kit (Werfen Company). nAPCsr, based on the activated partial thromboplastin time (APTT), was measured with the chromogenix coatest (Werfen Company) by dividing the APTT sample with exogenous activated protein C by the APTT without exogenous activated protein C sample. All samples were measured with the Instrumentation Laboratory ACL TOP 700 hemostasis testing system (Werfen Company) and did not receive any pretreatment besides thawing. Results of FII, FIX, FXI, free protein S and protein C measurements are expressed in IU/dL, while fibrinogen levels are expressed in g/L. The APCr was deduced from the nAPCsr as obtained via the Chromogenix coatest, with a lower ratio implying an increased APCr. For FII, FIX, FXI, free protein S, and protein C, re-measurement was done in case of a value of 180% or higher, as values above 180% could not be extrapolated and therefore become unreliable. Hematocrit at both time points was available for 93 (47%) of the participants; all other measurements were available for all participants at both time points. Of note, none of the patients received phlebotomy during the 12-month follow-up.

In addition, in part of the study population (n = 61, 31%) SHBG (measured by immuno-assay) levels at baseline and at 12 months after start of GAHT were available.

2.3 | Statistical analysis

Means and standard deviations (SD) were reported for normally distributed data and medians with interquartile range (IQR) for non-normally distributed data. Absolute and relative mean paired differences with 95% confidence interval (CI) between baseline values and after 12 months of GAHT treatment were estimated for the measurements of coagulation. Linear regression models were performed to estimate absolute differences with 95% CI of changes in these measurements over the treatment period (change in measures between 12 months of treatment and baseline) by route of administration. Transdermal estrogen was compared to oral estrogen in transwomen, and transdermal testosterone was compared to intramuscular testosterone in transmen. Next, we included age in the linear regression model to investigate potential interaction between age and the change in values of the measurements of coagulation. Last, similarly to with the measurements of coagulation, we estimated mean differences in SHBG levels before and 12 months after start of GAHT. In addition, to explore whether SHBG levels were associated with the change in coagulation profiles, we used a scatterplot and linear regression models to describe the association between the change in SHBG levels and change in APCr.

TABLE 1 Baseline characteristics of the study population

Clinical characteristics		
Group	**Transwomen**	**Transmen**
Total persons, *n*	98	100
Age in years, mean (SD)	33.7 (12.9)	26.9 (9.7)
Body mass index kg/m^2, mean (SD)	24.0 (4.6)	25.5 (5.5)
Height in cm, mean (SD)	180 (6.5)	167 (6.7)
Current smoker, *n* (%)	26 (26.5%)	37 (37.0%)
Alcohol user, *n* (%)	48 (49%)	49 (49.0%)
Hormone therapy		
Estrogen, oral *n* (%)	47 (48%)	NA
Estrogen, transdermal *n (%)*	46 (47%)	NA
Anti-androgen therapy combined with estrogen, oral *n* (%)	91 (93%)	NA
Anti-androgen monotherapy, oral *n* (%)	5 (5%)	NA
Testosteron, transdermal *n* (%)	NA	41 (41%)
Testosteron, intramuscular *n* (%)	NA	59 (59%)

Abbreviations: NA, not applicable; SD, standard deviation.

3 | RESULTS

3.1 | Study population

A total of 198 transpersons were included in the analyses, with 98 transwomen and 100 transmen. The clinical characteristics of the study participants are presented in Table 1. The mean age of the transwomen was 33.7 (SD 12.9). They had a mean body mass index (BMI) of 24.0 (SD 4.6), 26 (26.5%) were current smokers, and 48 (49%) used alcohol. Forty-six (47%) out of the 98 transwomen were treated with transdermal estradiol (Systen® 100 mg twice a week), while 47 (48%) were treated with oral estradiol valerate (Progynova® 2 mg twice a day). In 91 transwomen, estradiol was combined with the

oral anti-androgen, cyproterone acetate (Androcur® 50 mg daily). Five (5%) received anti-androgen therapy only, and were excluded from further analyses.

For the transmen, the mean age was 26.9 (SD 9.7). Their mean BMI was 25.5 (SD 5.5), 37 (37.0%) were smokers, and 49 (49.0%) used alcohol. Of the transmen, 59 (59%) out of 100 received intramuscular testosterone (Sustanon® 250 mg per 2 weeks or Nebido® 1000 mg per 2 weeks) and 41 (41%) received transdermal testosterone (Androgel® 50 mg per day). During the 12 months of follow-up in the study, no thrombotic events were observed.

3.2 | Difference in measurements of coagulation after 12 months of hormone therapy

The absolute and relative paired mean differences in measures of coagulation after 12 months of GAHT are shown in Table 2. In transwomen, notable procoagulant changes were a mean increase in FIX of 9.6 IU/dL (95% CI 3.1–16.0) and 13.5 IU/dL (95% CI 9.5–17.5) in FXI. Protein C decreased on average by −7.7 IU/dL (95% CI −10.1 to −5.2). Fibrinogen increased with 0.10 g/L (95% CI −0.04–0.24). Changes in the anticoagulant direction were an increase in APC ratio of 0.15 (95% CI −0.01–0.31), a decrease in haematocrit of −0.03 L/L (95% CI −0.04 to −0.02), and increase in free protein S of 2.5 IU/dL (95% CI −0.7–5.7). There were no apparent changes in FII levels. The relative differences, including the procoagulant or anticoagulant direction of these changes, are shown in Figure 1A.

In transmen, procoagulant changes were a mean increase in FIX of 7.9 IU/dL (95% CI 2.2–13.6) and an increase in hematocrit of 0.06 L/L (95% CI 0.05–0.07). Anticoagulant changes were a mean increase in APC ratio of 0.48 (95% CI 0.34–0.61), decrease in FII of −5.7 IU/dL (95% CI −9.9 to −1.5) and decrease in FXI of −7.7 IU/dL (95% CI −11.8 to −3.7). Protein S increased on average with 7.6 IU/dL (95% CI 1.6–13.6). Fibrinogen and protein C levels did not apparently change. The relative differences are shown in Figure 1B.

3.3 | Differences by route of administration

The results of the linear regression model for differences in measurements of coagulation by route of administration are shown in Table 3. The mean change after 12 months of treatment was higher for fibrinogen levels, 0.22 g/L (95% CI −0.06–0.51) and free protein S levels: 6.5 IU/dL (95% CI 0.3–12.8) in the group receiving oral estradiol than in the group receiving transdermal estrogen. Mean differences in FIX were lower in the oral estradiol group, −11.6 IU/dL (95% CI −24.4–1.2) than in the group receiving transdermal estradiol. There were no apparent differences in the other measurements. The relative differences in the measurements of coagulation are shown separately for transwomen receiving transdermal (Figure 1C) and oral estradiol (Figure 1E).

In transmen, mean differences in fibrinogen and protein C levels were lower in the group receiving intramuscular testosterone, −0.27 g/L (95% CI −0.65–0.10) and −7.7 IU/dL (−15.2 to −0.2),

TABLE 2 Absolute and relative paired mean differences in levels of coagulation markers 12 months after commencing hormone therapy compared to baseline levels

-	Mean (SD) at baseline	Mean (SD) at 12 months	Absolute mean paired difference (95% CI)	Relative change
Transwomen (*n* = 93)				
APCr ratio	3.06 (0.51)	3.21 (0.67)	0.15 (−0.01 to 0.31)	+4.9%
Factor II (IU/dL)	100.8 (14.8)	100.8 (18.9)	0.0 (−3.0 to 3.0)	0%
Factor IX (IU/dL)	122.2 (35.1)	131.7 (24.4)	9.6 (3.1 to 16.0)	+7.8%
Factor XI (IU/dL)	117.4 (26.2)	131.0 (22.6)	13.5 (9.5 to 17.5)	+11.6%
Fibrinogen (g/L)	3.06 (0.87)	3.17 (0.69)	0.10 (−0.04 to 0.24)	+3.6%
Hematocrit (L/L)	0.45 (0.03)	0.42 (0.02)	−0.03 (−0.04 to −0.02)	−6.7%
Protein S, free (IU/dL)	106.1 (17.7)	108.6 (20.8)	2.5 (−0.7 to 5.7)	+2.4%
Protein C (IU/dL)	108.2 (22.5)	100.6 (20.5)	−7.7 (−10.1 to −5.2)	−7.1%
Transmen (*n* = 100)				
APC ratio	2.84 (0.42)	3.32 (0.70)	0.48 (0.34 to 0.61)	+16.9%
Factor II (IU/dL)	102.4 (19.4)	96.7 (20.7)	−5.7 (−9.9 to −1.5)	−5.6%
Factor IX (IU/dL)	119.6 (27.4)	127.6 (29.9)	7.9 (2.2 to 13.6)	+6.6%
Factor XI (IU/dL)	125.5 (21.5)	117.8 (19.9)	−7.7 (−11.8 to −3.7)	−6.2%
Fibrinogen (g/L)	3.20 (0.92)	3.18 (0.88)	−0.02 (−0.20 to 0.17)	−0.6%
Hematocrit (L/L)	0.41 (0.03)	0.47 (0.03)	0.06 (0.05 to 0.07)	+14.6%
Protein S, free (IU/dL)	104.5 (28.9)	112.1 (25.4)	7.6 (1.6 to 13.6)	+7.3%
Protein C (IU/dL)	105.5 (26.5)	103.7 (19.9)	−1.9 (−5.6 to 1.9)	−1.8%

Abbrevations: APCr; activated protein C resistance; CI; confidence interval; SD; standard deviation.

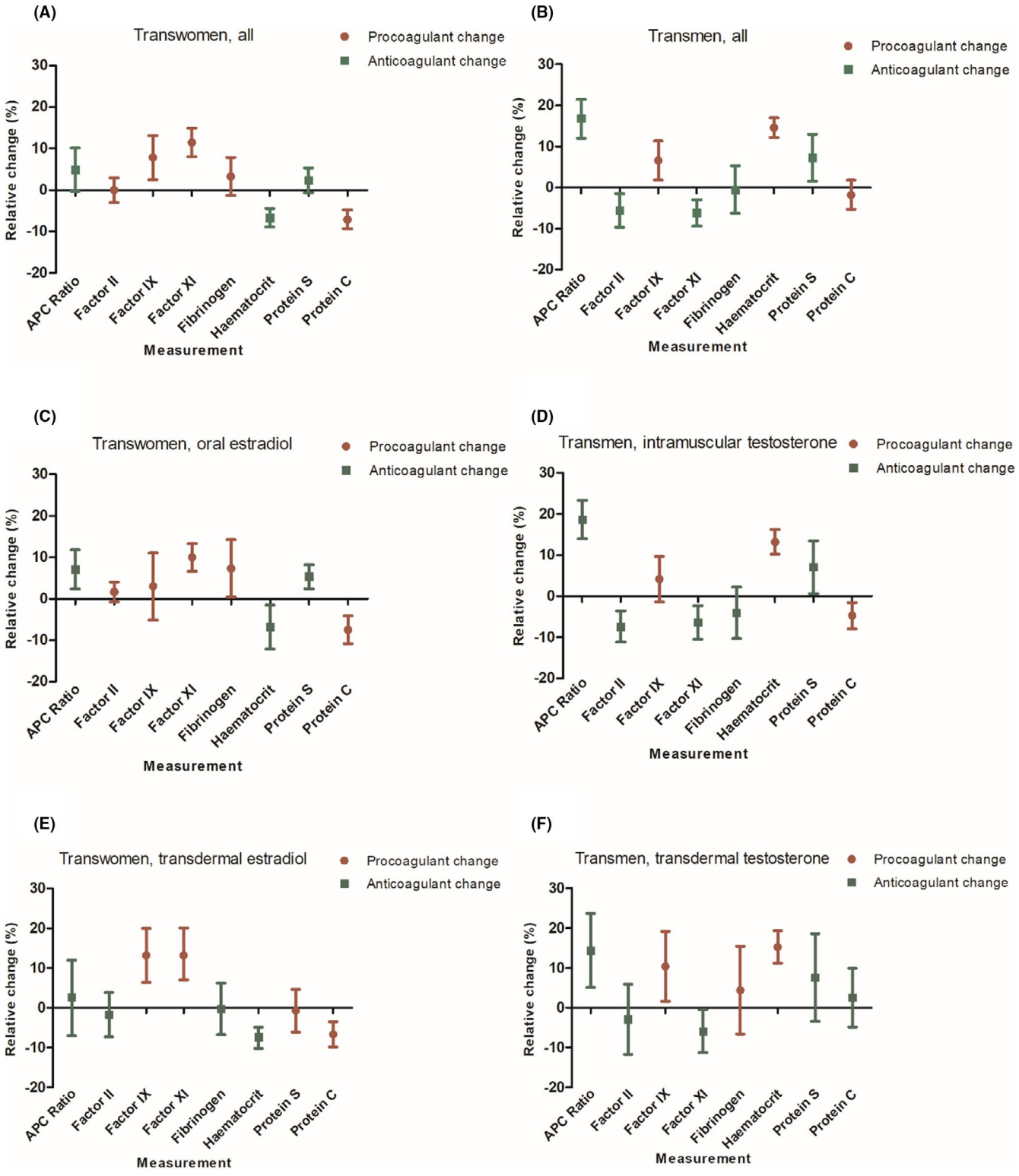

FIGURE 1 Relative change in the measures of coagulation for transwomen and transmen by routes of administration. This figure depicts the relative change (%) in the measures of coagulation for transwomen and transmen and for the routes of administration, separately. A red circle indicates a procoagulant direction of the effect of the change in measurement, where a green square indicates an anticoagulant direction of the effect. Anchored lines indicate 95% confidence intervals. Shown for the total group of transwomen (A) and transmen (B), seperate for transwomen which used oral estradiol (C) or transdermal estradiol (E) and seperate for transmen which used intramuscular testosterone (D) and transdermal testosterone (F).

TABLE 3 Linear regression coefficients with 95% confidence intervals for the absolute difference between measurements after 12 months of treatment and baseline measurements

-		Transwomen (*n* = 93), transdermal vs. oral estradiol; transdermal as reference	Transmen (*n* = 100), transdermal vs. intramuscular testosterone; transdermal as reference
APCr ratio	Administration route	0.1 (−0.2 to 0.5)	0.12 (−0.14 to 0.39)
	Adjusted for age	0.1 (−0.3 to 0.4)	0.16 (−0.11 to 0.44)
Factor II (IU/dL)	Administration route	3.4 (−2.5 to 9.3)	−4.8 (−13.4 to 3.7)
	Adjusted for age	1.7 (−4.6 to 8.0)	−6.3 (−15.0 to 2.4)
Factor IX (IU/dL)	Administration route	−11.6 (−24.4 to 1.2)	−7.0 (−18.6 to 4.5)
	Adjusted for age	−12.6 (−26.3 to 1.2)	−7.6 (−19.5 to 4.4)
Factor XI (IU/dL)	Administration route	−3.0 (−11.1 to 5.0)	−0.4 (−8.7 to 7.8)
	Adjusted for age	−3.8 (−12.4 to 4.8)	−1.0 (−9.5 to 7.5)
Fibrinogen (g/L)	Administration route	0.22 (−0.06 to 0.51)	−0.27 (−0.65 to 0.10)
	Adjusted for age	0.27 (−0.04 to 0.57)	−0.27 (−0.66 to 0.12)
Hematocrit (L/L)	Administration route	0.004 (−0.019 to 0.026)	−0.008 (−0.028 to 0.012)
	Adjusted for age	0.005 (−0.019 to 0.028)	−0.005 (−0.026 to 0.016)
Protein S, free (IU/dL)	Administration route	6.5 (0.3 to 12.8)	−0.2 (−12.4 to 12.0)
	Adjusted for age	6.7 (−0.1 to 13.4)	−1.7 (−14.3 to 11.0)
Protein C (IU/dL)	Administration route	−1.0 (−6.0 to 3.9)	−7.7 (−15.2 to −0.2)
	Adjusted for age	−3.1 (−8.2 to 2.1)	−8.2 (−16.0 to −0.5)

Abbreviation: APCr, activated protein C resistance.

respectively, than in the group receiving transdermal testosterone. There were no apparent differences in the other measurements. The relative differences in measurements of coagulation are shown separately for the transdermal group (Figure 1D) and intramuscular group (Figure 1F).

3.4 | Interaction with age

For both transwomen and transmen, there seemed to be an interaction with age to some extent for most measurements (Table 3). This was most profound for measurements of FII, FXI, and protein C. This indicates that the magnitude of change after 12 months of treatment is influenced by age.

3.5 | Sex-hormone-binding globulin levels and activated protein C resistance

In 23 (25%) of the transwomen and 38 (38%) of the transmen, a SHBG measurement was available both at baseline and after 12 months of follow-up. In both transmen and transwomen, the SHBG level was lower at 12 months follow-up with a mean paired difference of −5.4 mmol/L (95% CI −11.0–0.1) in the transwomen, and −20.9 (95% CI −35.8 to −5.9) in the transmen (Table S1). In transwomen, the change in SHBG levels after commencing hormone treatment seemed to be associated to some extent with the change in APCr (Figure S1). In the linear regression model, an increase in SHBG levels in transwomen was associated with a mean decrease in APCr, β −0.10 (95% CI −0.23 to −0.02). In transmen there was no association between change in SHBG levels and change in APCr, β 0.00 (95% CI −0.01–0.00).

4 | DISCUSSION

In this study, we investigated the effect of 12 months of GAHT on measurements of coagulation in transwomen and transmen. In transwomen, the changes were overall procoagulant with a substantial increase in FIX, FXI, and fibrinogen, and a decrease in protein C levels. In transmen, there was no apparent procoagulant shift. Interestingly, in both transmen and transwomen the APCr increased, typically indicating an overall lower procoagulant tendency. In transwomen, this might be explained by the decrease in protein C and increase in protein S, which may have influenced the measurement result.[31,32] Regarding the route of administration, in transwomen receiving oral estradiol, the change in fibrinogen and free protein S levels was larger and in FIX levels smaller, than in those receiving transdermal estradiol. The differences for type of administration of testosterone in transmen were small and most notably concerned decrease of fibrinogen levels and protein C for the intramuscular compared to the transdermal route. For both transwomen and transmen there was a mostly modest interaction between age and the observed difference in most of the measurements of coagulation. Both in transwomen and transmen the mean SHBG level was lower after commencing GAHT. In transwomen, a higher SHBG level after

12 months was associated with a lower APCr after 12 months than at baseline.

One of the most remarkable changes observed between the two groups was that of FXI levels, which clearly increased in transwomen and decreased in transmen after start of GAHT. Of interest, at baseline levels were higher in transmen than in the transwomen, mean 125.5 IU/dL (SD ±21.5) and mean 117.4 IU/dL (SD ±26.2), respectively. It seems that GAHT for transwomen approximates FXI levels of women (and vice versa for GAHT in transmen, which approximates FXI levels of men). Higher FXI levels are associated with both increased risk of first and recurrent VTE and the increase observed in transwomen after commencing GAHT may contribute to the observed increased VTE risk as described in the literature.[20-22,33,34]

Overall, use of estrogens (and anti-androgens) in transwomen resulted in an overall more procoagulant profile after 12 months, in line with observations in women who have an increase in estrogen and progestogen levels through oral contraceptive use, postmenopausal HT, or during pregnancy.[6-9,13,35] Thus, exogenous estradiol (with cyproterone acetate) seems to exert procoagulant effects in both persons with female and male sex assigned at birth. In transmen the introduction of testosterone did not result in a more procoagulant profile. From an evolutionary perspective these observations are plausible. As for the underlying mechanism, a more procoagulant coagulation profile due to increasing estrogen (and progestogen) levels during pregnancy is beneficial as it could reduce blood loss during labor.[36] For testosterone, the evolutionary benefit of a similar procoagulant change is less evident. As procoagulant changes are associated with VTE risk, the results of our study are of clinical relevance. In transmen, in line with a previous study,[37] the only potential procoagulant change seemed to be an increase in hematocrit after initiating testosterone therapy. However, the causal role of hematocrit in VTE is currently debated.[38] In addition, overall, there were only small differences in effect between the intramuscular and transdermal route. Thus, these findings appear reassuring regarding the risk of VTE in transmen using testosterone, although no definite conclusions can be drawn.

We observed a more procoagulant profile in transwomen after 12 months of HT. These results are in line with the limited available literature, in which the incidence of VTE appears higher in transwomen than in men of the same age.[2,20-22] In transwomen receiving oral preparations the difference in fibrinogen and free protein S was larger and the change in FIX was smaller than in those receiving transdermal preparations. Overall, these findings suggest a more procoagulant change with the oral preparations compared to the transdermal form, although relatively small. The differences between these routes of administration, in terms of change in coagulation profiles, do not appear as substantial in the reports concerning high-dosed oral estrogen in which substantial differences in procoagulant changes and VTE risk were observed for oral formulations compared to transdermal preparations.[10,24,25] It should be mentioned that although various measurements of coagulation are associated with VTE risk,[39] studies that assess the absolute incidence of objectively confirmed symptomatic VTE remain the gold standard. To draw definite conclusions on risk of VTE in transwomen and transmen, adequately sized robust (cohort) studies with appropriate control groups (cismen for transwomen and ciswomen for transmen) are needed. Ideally, as a next step risk assessment models are then developed to inform transpersons and their physicians on the absolute risk of VTE with use of (different types of) HT, in this way aiding informed decision making.[40] In this context, in transwomen, the change in SHBG levels after commencing GAHT may be an important predictor of a procoagulant change. In line with studies on ciswomen commencing oral contraceptives, increased SHBG levels seem to reflect a more "estrogenic" and procoagulant state, which is associated with an observed increased risk of VTE. Further studies are needed to assess whether the change in SHBG in transwomen is a predictor of VTE events.

This study has several important strengths. We were able to investigate a broad scope of the coagulation system in a large group of both transwomen and transmen. Moreover, the design of the study allowed pairwise estimation of the effects (i.e., every person is their own control), eliminating the potential effects of fixed confounders (e.g., genetics, body height, age, etc,).

When interpreting the results, it must be noted that we only investigated a selection of parameters of coagulation, on which changes have been described in women after commencing oral contraceptives. Hence, we did not measure other procoagulant or anticoagulant changes that may have taken place *in vivo*. In addition, although we observed procoagulant changes in some parameters, and anticoagulant changes in others, it is unknown how these can be placed in context with each other and whether they actually sum up to a more procoagulant or anticoagulant state. However, the findings of our study are in line with a recent, albeit smaller, study on the effect of estrogen use in transgender women (n = 26) on more global coagulation assays (i.e., thromboelastography, thrombin generation, and plasma fibrin generation). In this study, more global procoagulant profiles were observed in the transgender women using estrogens compared to men (n = 55).[41] In addition, all measurements are subject to both biological and analytical variation, which may have influenced the results. This variation is reflected in the 95% CI of the effect estimates, and could even have led to an underestimation of the results. Last, given the existing evidence on hormone use and changes in coagulation parameters, the changes we observed are likely an effect of the hormone therapy. However, we cannot exclude the possibility that other influences related to the transition trajectory (e.g., changes in BMI or smoking habits) have affected the measured parameters of coagulation.[42] In addition, several other limitations of the study should be kept in mind when interpreting its results. First, our study was aimed at providing mechanistic insight and therefore we investigated a broad range of measurements of coagulation; however, as mentioned, procoagulant changes do not necessarily cause VTE. In addition, we did not investigate the type of intramuscular testosterone (Sustanon® or Nebido®) separately due to a small number of Nebido® users. Last, nearly all transwomen received both estradiol and cyproterone acetate,

therefore we have observed the average effect on coagulation of both. Combined with estradiol for use as contraceptive pill in women, cyproterone acetate is associated with a higher risk of VTE compared to other hormonal contraceptive preparations.[43] A small study reported the most profound procoagulant changes in transwomen using estradiol with cyproterone acetate compared to transwomen who used cyproterone acetate only.[23] Further studies are needed to investigate the effects of estradiol and cyproterone acetate separately.

In conclusion, coagulation profiles in transwomen and transmen were influenced by 12 months of GAHT use and affected by route of administration and age to some extent. Testosterone use in transmen did not result in apparent procoagulant changes which, in context of the available literature, provides some reassurance regarding the risk of VTE. In transwomen use of estradiol and anti-androgens resulted in overall more procoagulant profiles, which likely contributes to the observed increased risk of VTE in this population.

ACKNOWLEDGMENT

We want to express our gratitude to all participants and persons involved in the study.

CONFLICTS OF INTEREST

LJJS: reports no conflicts of interest related to this work. NLDS: reports no conflicts of interest related to this work. NMN: reports no conflicts of interest related to this work. JJKD: reports no conflicts of interest related to this work. SSC: reports no conflicts of interest related to this work. MDH: reports no conflicts of interest related to this work.

AUTHOR CONTRIBUTIONS

L.J.J.S, N.L.D.S., S.C.C., and M.D.H. designed the analyses. L.J.J.S., N.L.D.S., N.M.N., J.J.K.D., and M.D.H. collected data for the analyses. L.J.J.S. performed the analyses. L.J.J.S. wrote the manuscript. N.L.D.S., N.M.N., J.J.K.D., S.C.C., and M.D.H. critically revised the manuscript. All authors approved of the final version of the manuscript.

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REFERENCES

1. Kyrle PA, Minar E, Bialonczyk C, Hirschl M, Weltermann A, Eichinger S. The risk of recurrent venous thromboembolism in men and women. *N Engl J Med*. 2004;350:2558-2563.
2. Naess IA, Christiansen SC, Romundstad P, Cannegieter SC, Rosendaal FR, Hammerstrøm J. Incidence and mortality of venous thrombosis: a population-based study. *J Thromb Haemost*. 2007;5:692-699.
3. Hendriksen JMT, Koster-van Ree M, Morgenstern MJ, et al. Clinical characteristics associated with diagnostic delay of pulmonary embolism in primary care: a retrospective observational study. *BMJ Open*. 2017;7:e012789.
4. Scheres LJJ, Brekelmans MPA, Beenen LFM, Büller HR, Cannegieter SC, Middeldorp S. Sex-specific differences in the presenting location of a first venous thromboembolism. *J Thromb Haemost*. 2017;15:1344-1350.
5. Speed V, Roberts LN, Patel JP, Arya R. Venous thromboembolism and women's health. *Br J Haematol*. 2018;183:346-363.
6. Rosing J, Tans G. Effects of oral contraceptives on hemostasis and thrombosis. *Am J Obstet Gynecol*. 1999;180:S375-382.
7. Middeldorp S, Meijers JC, van den Ende AE, et al. Effects on coagulation of levonorgestrel- and desogestrel-containing low dose oral contraceptives: a cross-over study. *Thromb Haemost*. 2000;84:4-8.
8. Tans G, Curvers J, Middeldorp S, et al. A randomized cross-over study on the effects of levonorgestrel- and desogestrel-containing oral contraceptives on the anticoagulant pathways. *Thromb Haemost*. 2000;84:15-21.
9. Shatzel JJ, Connelly KJ, DeLoughery TG. Thrombotic issues in transgender medicine: A review. *Am J Hematol*. 2017;92:204-208.
10. Vehkavaara S, Silveira A, Hakala-Ala-Pietilä T, et al. Effects of oral and transdermal estrogen replacement therapy on markers of coagulation, fibrinolysis, inflammation and serum lipids and lipoproteins in postmenopausal women. *Thromb Haemost*. 2001;85:619-625.
11. Koh KK, Mincemoyer R, Bui MN, et al. Effects of hormone-replacement therapy on fibrinolysis in postmenopausal women. *N Engl J Med*. 1997;336:683-690.
12. Grodstein F, Stampfer MJ, Goldhaber SZ, et al. Prospective study of exogenous hormones and risk of pulmonary embolism in women. *Lancet Lond Engl*. 1996;348:983-987.
13. Kroon UB, Silfverstolpe G, Tengborn L. The effects of transdermal estradiol and oral conjugated estrogens on haemostasis variables. *Thromb Haemost*. 1994;71:420-423.
14. Jick H, Derby LE, Myers MW, Vasilakis C, Newton KM. Risk of hospital admission for idiopathic venous thromboembolism among users of postmenopausal oestrogens. *Lancet Lond Engl*. 1996;348:981-983.
15. Martinez C, Suissa S, Rietbrock S, et al. Testosterone treatment and risk of venous thromboembolism: population based case-control study. *BMJ*. 2016;355:i5968.
16. Agledahl I, Brodin E, Svartberg J, Hansen J-B. Impact of long-term testosterone treatment on plasma levels of free TFPI and TF-induced thrombin generation ex vivo in elderly men with low testosterone levels. *Thromb Haemost*. 2009;102:945-950.
17. Brodin E, Vikan T, Hansen J-B, Svartberg J. Testosterone, hemostasis, and cardiovascular diseases in men. *Semin Thromb Hemost*. 2011;37:87-94.
18. Hembree WC, Cohen-Kettenis PT, Gooren L, et al. Endocrine treatment of gender-dysphoric/gender-incongruent persons: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2017;102:3869-3903.
19. Connors JM, Middeldorp S. Transgender patients and the role of the coagulation clinician. *J Thromb Haemost*. 2019;17:1790-1797.
20. Asscheman H, T'Sjoen G, Lemaire A, et al. Venous thromboembolism as a complication of cross-sex hormone treatment of male-to-female transsexual subjects: a review. *Andrologia*. 2014;46:791-795.
21. Getahun D, Nash R, Flanders WD, et al. Cross-sex hormones and acute cardiovascular events in transgender persons: a cohort study. *Ann Intern Med*. 2018;169(4):205.
22. de Nota NMWCM, Blok CJM, Gooren LJG, Kreukels BPC, den Heijer M. The occurrence of acute cardiovascular events in transgender individuals receiving hormone therapy: results from a large cohort study. *Circulation*. 2019;139(11):1461-1462.

23. Toorians AWFT, Thomassen MCLGD, Zweegman S, et al. Venous thrombosis and changes of hemostatic variables during cross-sex hormone treatment in transsexual people. *J Clin Endocrinol Metab*. 2003;88:5723-5729.
24. Asscheman H, Gooren LJ, Eklund PL. Mortality and morbidity in transsexual patients with cross-gender hormone treatment. *Metabolism*. 1989;38:869-873.
25. van Kesteren PJ, Asscheman H, Megens JA, Gooren LJ. Mortality and morbidity in transsexual subjects treated with cross-sex hormones. *Clin Endocrinol (Oxf)*. 1997;47:337-342.
26. Vinogradova Y, Coupland C, Hippisley-Cox J. Use of hormone replacement therapy and risk of venous thromboembolism: nested case-control studies using the QResearch and CPRD databases. *BMJ*. 2019;364:k4810.
27. Raps M, Helmerhorst F, Fleischer K, et al. Sex hormone-binding globulin as a marker for the thrombotic risk of hormonal contraceptives. *J Thromb Haemost*. 2012;10:992-997.
28. Dekker MJHJ, Wierckx K, Van Caenegem E, et al. A European network for the investigation of gender incongruence: endocrine part. *J Sex Med*. 2016;13:994-999.
29. van Hylckama Vlieg A, Helmerhorst FM, Vandenbroucke JP, Doggen CJM, Rosendaal FR. The venous thrombotic risk of oral contraceptives, effects of oestrogen dose and progestogen type: results of the MEGA case-control study. *BMJ*. 2009;339:b2921.
30. Roach REJ, Lijfering WM, Helmerhorst FM, Cannegieter SC, Rosendaal FR, van Hylckama Vlieg A. The risk of venous thrombosis in women over 50 years old using oral contraception or postmenopausal hormone therapy. *J Thromb Haemost*. 2013;11:124-131.
31. Kadauke S, Khor B, Van Cott EM. Activated protein C resistance testing for factor V Leiden. *Am J Hematol*. 2014;89:1147-1150.
32. de Ronde H, Bertina RM. Laboratory diagnosis of APC-resistance: a critical evaluation of the test and the development of diagnostic criteria. *Thromb Haemost*. 1994;72:880-886.
33. Meijers JC, Tekelenburg WL, Bouma BN, Bertina RM, Rosendaal FR. High levels of coagulation factor XI as a risk factor for venous thrombosis. *N Engl J Med*. 2000;342:696-701.
34. Kyrle PA, Eischer L, Šinkovec H, Eichinger S. Factor XI and recurrent venous thrombosis: an observational cohort study. *J Thromb Haemost*. 2019;17:782-786.
35. Scheres LJJ, Bistervels IM, Middeldorp S. Everything the clinician needs to know about evidence-based anticoagulation in pregnancy. *Blood Rev*. 2018;33:82-97.
36. Bremme KA. Haemostatic changes in pregnancy. *Best Pract Res Clin Haematol*. 2003;16:153-168.
37. Defreyne J, Vantomme B, Van Caenegem E, et al. Prospective evaluation of hematocrit in gender-affirming hormone treatment: results from European Network for the Investigation of Gender Incongruence. *Andrology*. 2018;6:446-454.
38. Schreijer AJM, Reitsma PH, Cannegieter SC. High hematocrit as a risk factor for venous thrombosis. Cause or innocent bystander? *Haematologica*. 2010;95:182-184.
39. Cushman M, O'Meara ES, Folsom AR, Heckbert SR. Coagulation factors IX through XIII and the risk of future venous thrombosis: the Longitudinal Investigation of Thromboembolism Etiology. *Blood*. 2009;114:2878-2883.
40. Scheres LJJ, Lijfering WM, Cannegieter SC. Current and future burden of venous thrombosis: Not simply predictable. *Res Pract Thromb Haemost*. 2018;2:199-208.
41. Lim HY, Leemaqz SY, Torkamani N, et al. Global coagulation assays in transgender women on oral and transdermal estradiol therapy. *J Clin Endocrinol Metab*. 2020;105:e2369.
42. Klaver M, de Mutsert R, Wiepjes CM, et al. Early hormonal treatment affects body composition and body shape in young transgender adolescents. *J Sex Med*. 2018;15:251-260.
43. Middeldorp S. Thrombosis in women: what are the knowledge gaps in 2013? *J Thromb Haemost*. 2013;11(Suppl 1):180-191.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Scheres LJ, Selier NL, Nota NM, Diemen JJ, Cannegieter SC, Heijer M. Effect of gender-affirming hormone use on coagulation profiles in transmen and transwomen. *J Thromb Haemost*. 2021;19:1029–1037. https://doi.org/10.1111/jth.15256