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Prospective evaluation of hematocrit in gender-affirming hormone treatment: results from European Network for the Investigation of Gender Incongruence

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SUMMARY

In trans persons on gender-affirming hormonal treatment, a decrease (in trans women) or increase (in trans men) in hematocrit is often observed. Reference ranges for evaluation of hematocrit levels in trans persons have not been established. This prospective cohort study is part of the European Network for the Investigation of Gender Incongruence (ENIGI). At the Ghent and Amsterdam sites, we included 625 hormone-naïve trans persons. Gender-affirming hormonal treatment was initiated at the first visit. In trans men, serum hematocrit (Hct) levels increased during the first year (+4.9 Hct %, 95% CI 3.82–5.25), with the most pronounced increase during the first 3 months (+2.7 Hct %, 95% CI 1.94–3.29). Trans men receiving testosterone esters had a larger increase in serum hematocrit levels compared to trans men receiving testosterone undecanoate (Δ 0.8 Hct %). Of 192 trans men, 22 (11.5%) developed serum hematocrit levels ≥50.0%. Trans men on testosterone undecanoate were less likely to develop hematocrit levels ≥50% or ≥52%, compared to trans men on testosterone esters, and were less likely to develop hematocrit levels ≥50%, compared to trans men on testosterone gel. In trans women, serum hematocrit had dropped by 4.1 Hct % (95% CI 3.50–4.37) after 3 months, after which only small decreases were observed. In conclusion, serum hematocrit levels can be found in the reference range of the perceived gender as from 3 months after the initiation of gender-affirming hormonal treatment.

INTRODUCTION

Gender dysphoria is defined as a marked incongruence between a person's birth-assigned gender and the experienced or expressed gender (American Psychiatric Association, 2013). Trans persons may request gender-affirming hormonal treatment to alleviate their gender dysphoria (Hembree *et al.*, 2017). This treatment aims to suppress the secondary sex characteristics of the birth-assigned gender and to induce the secondary sex characteristics of the experienced gender. In trans women, this is achieved through the administration of anti-androgens and estrogens. In trans men, exogenous testosterone is administered (Moore *et al.*, 2003). Frequent laboratory monitoring is recommended as part of the endocrinological follow-up for patient management and to detect possible adverse events (Hembree *et al.*, 2017). Known side effects of the administration of estrogens and/or anti-androgens include the occurrence of thromboembolic complications (Asscheman *et al.*, 2014), hyperprolactinemia (van Kesteren *et al.*, 1997), and hypertriglyceridemia (Elamin *et al.*, 2010). Testosterone treatment can be associated with erythrocytosis, hypertriglyceridemia, lowering of high-density lipoprotein cholesterol, salt retention, arterial hypertension, and acne (Moore *et al.*, 2003; Hembree *et al.*, 2017).

One of the most prominent changes seen in the routine laboratory monitoring of trans persons is a change in hematocrit and hemoglobin: a decrease in trans women (Schlatterer *et al.*, 1998; Roberts *et al.*, 2014; Quirós *et al.*, 2015; Tack *et al.*, 2017) and an increase in trans men (Schlatterer *et al.*, 1998; Jacobeit *et al.*,

2009; Chandra *et al.*, 2010; Mueller *et al.*, 2010; Pelusi *et al.*, 2014; Quirós *et al.*, 2015; Jarin *et al.*, 2017). As in hypogonadal men on testosterone treatment, the induction of erythrocytosis in trans men on testosterone treatment is of concern because it might be associated with an elevated thrombotic risk. Although the expected changes in hematocrit after initiation of gender-affirming hormonal treatment are mostly physiological, laboratory values during routine monitoring in the transition period will often be reported as anomalous because laboratories apply gender-specific reference intervals in accordance with the patient's legal gender. To date, it has not been established which reference ranges should be used for hematocrit levels in trans persons, which places these populations at a higher risk for diagnostic errors. Our primary study endpoint was to determine when changes in serum hematocrit levels occur and what the expected values of serum hematocrit levels are in trans persons on gender-affirming hormonal treatment. Furthermore, we aimed to assess whether testosterone treatment in trans men is safe and whether the mode of testosterone administration has an impact on erythrocytosis rates.

METHODS

The ENIGI study is a multicenter prospective cohort study conducted in four European treatment centers (Ghent, Oslo, Florence, and Amsterdam) (Dekker *et al.*, 2016). For the present substudy, data from Ghent and Amsterdam were selected due to logistical reasons. From February 2010 until October 2016, 1023 trans persons have been included in the Belgian–Dutch sample of the European Network for the Investigation of Gender Incongruence (ENIGI) study. A data lock was performed: only data that had already been entered in the database were analyzed (625 participants: 285 trans men and 340 trans women) (Fig. 1). All patients were at least 16 years old (in the Belgian cohort) or 17 years old (in the Dutch cohort) and underwent a standardized diagnostic procedure to confirm the diagnosis of gender dysphoria before initiating treatment (Dekker *et al.*, 2016). Patients were included in the ENIGI endocrine protocol when they started gender-affirming hormonal treatment. Every patient was treated in accordance with the World Professional Association for Transgender Health Standards of Care, edition 7 (Davies *et al.*, 2015). Exclusion criteria were previous use of gender-affirming hormones and insufficient knowledge of the native languages (Dutch or French). At the start of the study, patients received oral and written information about the ENIGI endocrine protocol. A written informed consent was obtained according to the institution's guidelines. Short-term follow-up currently consists of a baseline visit and subsequent visits after 3, 6, 9 and 12 months in Amsterdam and at baseline, 3, 6, 9, 12, 18, 24 and 36 months in Ghent. A venous blood sample was obtained upon each visit, independent of the time to testosterone administration.

In trans women, gender-affirming hormonal treatment consists of cyproterone acetate (Androcur®; Bayer, Diegem, Belgium) 50 mg once daily, combined with an oral estradiol agent, estradiol valerate (Progynova®; Bayer) 2 mg twice daily. In patients older than 45 years, estradiol is administered transdermally in the form of estradiol patches (Systen®; Janssen-Cilag, Breda, the Netherlands; and Dermestril®; Besins, Brussels, Belgium) in a dose of 50–100 μg/24 h, to avoid increased thrombotic risk associated with the administration of oral estrogens caused by the hepatic first-pass effect (Dekker *et al.*, 2016). In the case of non-tolerance, 2 mg of transdermal 17-β E2 gel twice daily (Oestrogel®; Besins) is given. After orchiectomy, estrogens alone are continued in an unchanged dose. In Ghent, trans men receive intramuscular long-acting testosterone (Nebido® 1000 mg once every 12 weeks). In Amsterdam, trans men can choose between testosterone gel in a daily dose of 50 mg or intramuscular administration, either as testosterone esters (Sustanon® 250 mg every 2 weeks) or as testosterone undecanoate (Nebido® 1000 mg every 12 weeks).

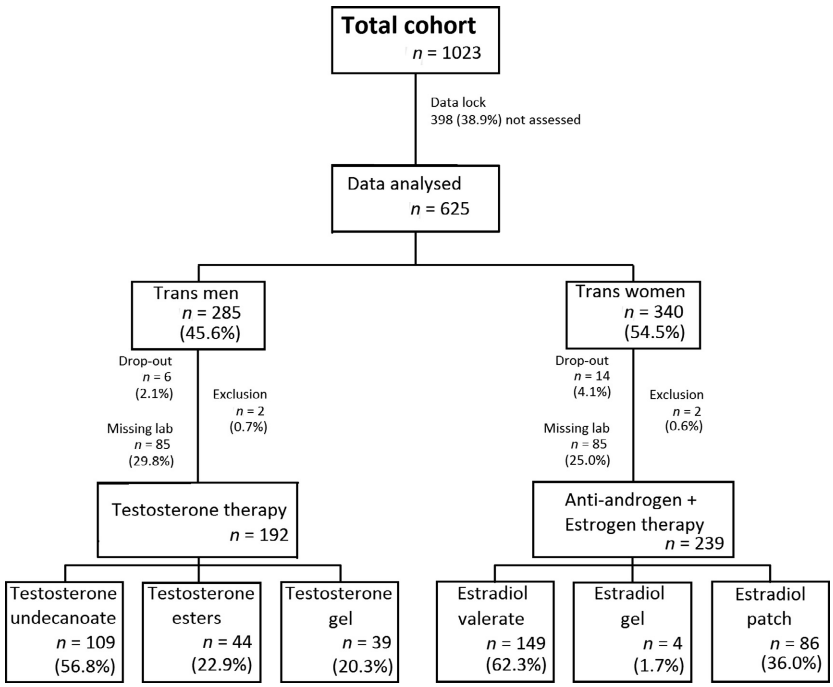
In Ghent, hematocrit was measured using a Sysmex XE-5000 (Sysmex Corporation, Kobe, Japan), with an interassay CV of <1.4%. Competitive chemiluminescent immunoassays were run for estradiol (E170 Modular, Roche, Gen III, LOQ 25 pg/mL, interassay CV 3.2%), serum testosterone (E170 Modular, Roche, Gen II, LOQ 10 ng/dL (0.4 nmol/L), interassay CV 2.6%), LH (E170 Modular, Roche, Gen III, interassay CV 3.48%, LOQ 0.1 mIU/mL), and FSH (E170 Modular, Roche, Gen III, interassay CV 3.3%, LOQ 0.1 mIU/mL), and for SHBG, a sandwich-type chemiluminescent immunoassay was employed (E170 Modular, Roche, Gen III, interassay CV 4.06%, LOQ 0.35 mIU/mL).

Before March 19, 2015, estradiol was measured using an E170 Modular (Gen II; Roche Diagnostics, Mannheim, Germany). For conversion of estradiol values measured before March 19, 2015, the formula Gen III = 6.687940 + 0.834495 * Gen II was used (E170 Modular; Roche Diagnostics, Mannheim, Germany).

In Amsterdam, hematocrit was measured using a CELL-DYN Sapphire (Abbott Diagnostics, Lake Forest, IL, USA) with an interassay CV of 1.1%. Estradiol was also measured using an E170 Modular (Gen II; Roche Diagnostics) until March 19, 2015. Thereafter, estradiol was measured using an E170 Modular (Gen III; Roche Diagnostics); the same conversion formula as in Ghent was used to convert estradiol values. Testosterone was measured using a radioimmunoassay (RIA) (Coat-A-Count; Siemens, Los Angeles, CA, USA) with an interassay CV of 7–20% and a LOQ of 1 nmol/L until January 2013. Thereafter, testosterone was measured using competitive immunoassay (Architect; Abbott, Abbott Park, IL, USA) with an interassay CV of 6–10% and a LOQ of 0.1 nmol/L. RIA values were converted to competitive immunoassay values. For testosterone levels below 8 nmol/L, the formula Architect = 1.1 * RIA + 0.2 was used; for testosterone levels above 8 nmol/L, the formula Architect = 1.34 * RIA – 1.65 was used. LH, FSH, and SHBG were measured using chemiluminescent microparticle immunoassay (Architect System; Abbott), with an interassay CV of 4% and a LOQ of 2 U/L for LH, FSH, and SHBG.

Data were analyzed using IBM SPSS 24.0 (SPSS, Chicago, IL, USA). Data were verified for normal distribution using the Shapiro–Wilk test. Differences between groups were analyzed by unpaired Student's t-test (normally distributed data) and the Mann–Whitney *U*-test (non-normally distributed data). To evaluate hematocrit differences in time, a mixed model was applied to the outcome variable hematocrit, with visit (number of months) as fixed factor and with a random intercept for baseline serum hematocrit levels. For trans men, type of testosterone was used as a factor, and serum testosterone and SHBG levels were used as covariates. Serum levels of estradiol, follicle-stimulating hormone (FSH), luteinizing hormone (LH), free testosterone, baseline hematocrit*visit (interaction), BMI, smoking, recreational drugs, and alcohol habits were tested but not included as

Figure 1 Flowchart of the study population.



factors/covariates. For trans women, serum levels of testosterone were used as a factor. Serum levels of estradiol, SHBG, FSH, LH, free testosterone, baseline hematocrit*visit (interaction), type of estrogen treatment, BMI, smoking, recreational drugs, and alcohol habits were tested but not included as factors/covariates. For both trans men and trans women, center was not included in the model, as mean hematocrit levels did not differ at any given visit. In addition, when used in the linear mixed models, SPSS Statistics labeled this category as 'redundant'.

RESULTS

Data were analyzed from 625 trans persons that had been included in the ENIGI study between February 2010 and October 2016 (Fig. 1). Four trans persons (two trans men and two trans women) were excluded from the analysis because of treatment with testosterone esters instead of testosterone undecanoate in Ghent ($n = 1$), delayed initiation of estrogen treatment ($n = 1$), use of estrogens only without administration of an anti-androgen ($n = 1$), and gastric bypass ($n = 1$). After applying exclusion criteria and omitting dropouts and missing laboratories, 192 trans men and 239 trans women were included in the analysis. Baseline characteristics of the study population are shown in Table 1.

In the Amsterdam cohort, two trans women experienced a thromboembolic event during gender-affirming hormonal treatment. One 23-year-old trans woman experienced a cerebrovascular accident 16 months after the initiation of gender-affirming hormonal treatment, and one 59-year-old trans woman experienced thrombophlebitis 18 months after the initiation of gender-affirming hormonal treatment. None of the trans men experienced a thromboembolic event during gender-affirming hormonal treatment.

When serum hormone levels were measured after 3 months of gender-affirming hormonal treatment, the mean testosterone level in trans men was 491 ng/dL [289.5–655.3], while the mean testosterone level in trans women had decreased to 20.3 ng/dL [14.5–29.0] (Table 2). These values are within the normal reference ranges for testosterone in cisgender men and women, respectively, thus indicating effective testosterone treatment in trans men and effective androgen deprivation in trans women. As can be expected, estradiol levels in trans women were significantly higher 3 months after treatment had started (Table 2). Oophorectomy did not have an impact on serum testosterone and estradiol levels in trans men ($n = 34$, 41.5% ($p = 0.137$) at 18 months; $n = 57$, 85.1% ($p = 0.258$) at 24 months; and $n = 19$, 95.0% ($p = 1.000$) at 36 months). Serum testosterone levels were not significantly different between trans men on testosterone undecanoate, testosterone esters, or testosterone gel ($p = 0.387$).

In trans women, serum hematocrit levels had dropped by 4.1 Hct % (95% CI 3.50–4.37, $p < 0.001$) after the first 3 months of gender-affirming hormonal treatment (from 45.1% [42.7–47.5] at baseline to 41.0% [39.9–43] at 3 months), after which only smaller further decreases were observed (Fig. 2a and Table 2). No further differences in serum hematocrit levels occurred after 18 months of gender-affirming hormonal treatment in trans women. In addition, estrogen mode of administration did not influence serum hematocrit levels ($p = 0.864$). In trans women, a positive correlation between serum hematocrit levels and serum testosterone levels ($r = 0.501$, $p < 0.001$) and a negative correlation between serum hematocrit levels and serum estradiol levels ($r = 0.085$, $p = 0.005$) were observed.

In trans men, serum hematocrit levels gradually increased during the first 12 months (+4.9 Hct %, 95% CI 3.82–5.25, $p < 0.001$), with the most pronounced increase during the first 3 months (+2.7 Hct %, 95% CI 1.94–3.29, $p < 0.001$) (Fig. 2a and Table 2). After reaching peak levels at 12 months (46.0% [44.0–47.0]), we found a 1 Hct % decrease in hematocrit at 18 months (95% CI 0.51–2.30, $p < 0.001$). There were no statistically

Table 1 Baseline characteristics of the study population

			Trans men (*N* = 192)	Trans women (*N* = 239)	*p*-value
Hematocrit (%) (range)			41.1 ± 3.0 (30.2–49.9)	45.0 ± 2.5 (35.3–50.0)	<0.001*
Age (years) (range)			22.5 [20–31] (17–62)	28.5 [22–43] (16–69)	<0.001*
Weight (kg) (range)			65.0 [57.1–73.0] (46.4–125.0)	72.8 [65.4–84.0] (49.9–137.2)	<0.001*
Length (m) (range)			1.66 ± 7.40 (1.32–1.83)	1.79 ± 6.70 (1.62–1.97)	<0.001*
BMI (kg/m^2)			24.35 ± 4.80 (17.46–38.22)	23.35 ± 4.50 (14.11–41.72)	<0.001*
Units alcohol/week (range)			[0.0–2.0] (0.0–25)	0.0 [0.0–3.0] (0.0–35)	0.162
Smoking	Number of smokers (*n*, %)		50 (26.0%)	50 (21.9%)	0.406
	Missing (*n*, %)		27 (14.1%)	38 (16.7%)	0.500
	Number of cigarettes smoked/day (range)		8.0 [4.0–15.0] (1–25)	10.0 [3.5–19.0] (1–90)	0.254
Type of gender-affirming hormonal treatment	CPA 50 mg + estrogens	EV 2 × 2 mg	/	149 (62.3%)	
		EG 2 × 1.5 mg		4 (1.7%)	
		TD 100 μg/72 h		85 (35.6%)	
		TD 50 μg/72 h		1 (0.4%)	
	Testosterone	TU	109 (56.8%)		
		TE	44 (22.9%)		
		TG	39 (20.3%)		

Baseline characteristics of the study population, for trans men and trans women. Tests for normality were performed using the Shapiro–Wilk test. For normally distributed values, mean values ± standard deviation are shown. For values that are not normally distributed, median values and IQR [P25 and P75] are shown, unless otherwise specified. For normally distributed values, unpaired Student's t-test was used to quantify differences between both groups, and for nonparametric values, the Mann–Whitney *U*-test was performed. Statistically significant differences between both groups are marked with * ($p < 0.05$). CPA, cyproterone acetate; EV, estradiol valerate; EG, estradiol gel; TD, estradiol transdermal patch; TU, testosterone undecanoate; TE, testosterone esters; TG, testosterone gel.

significant differences between hematocrit levels at 12, 24, or 36 months of follow-up. In trans men, higher serum hematocrit levels were associated with relatively higher serum testosterone levels ($r = 0.238$, $p < 0.001$) and lower serum SHBG ($r = -0.209$, $p < 0.001$) and estradiol ($r = -0.120$, $p = 0.001$) levels. Trans men receiving testosterone esters had a larger increase in serum hematocrit levels compared to trans men receiving testosterone undecanoate (Δ 0.8 Hct %) (Fig. 2b and Table 3).

At baseline, none of the trans men in our cohort had a serum hematocrit level >50.0%. After 3 months of gender-affirming hormonal treatment, eight trans men (4.3%) had a serum hematocrit level ≥50.0% (range 50.0–51.0%) (Table 4). In total, 22 of 192 trans men (11.5%) developed serum hematocrit levels ≥50.0% during the investigation period: 10 of 109 patients on testosterone undecanoate (9.2%), seven of 44 patients on testosterone esters (15.9%), and five of 39 patients on testosterone gel (12.8%). Trans men developing serum hematocrit levels >50% had a higher body mass index (BMI) (27.62 [24.67–31.91]), compared to trans men in whom hematocrit did not increase above 50% (24.50 [21.70–28.68], $p = 0.025$). There was no difference between both groups regarding smoking habit ($p = 0.181$), drinking habits ($p = 0.668$), the use of psychoactive medication ($p = 0.638$), or recreational drug use ($p = 1.000$). Four trans men developed serum hematocrit levels ≥52.0% (three on testosterone esters (6.8%) and one on testosterone gel (2.6%)). The maximum measured level of serum hematocrit was 54.0%. Trans men on testosterone undecanoate were less likely to develop hematocrit levels ≥50% or ≥52%, compared to trans men on testosterone esters ($p < 0.001$ and $p = 0.005$, respectively), and were less likely to develop hematocrit levels ≥50%, compared to trans men on testosterone gel ($p = 0.033$). There was no significant difference in erythrocytosis rates between trans men on testosterone esters and trans men on testosterone gel ($p = 0.454$).

DISCUSSION

Testosterone administration in trans men is associated with a rise in hematocrit, which occurs predominantly during the first year of gender-affirming hormonal treatment. The results in trans men we present here are in line with earlier observations (Schlatterer *et al.*, 1998; Jacobeit *et al.*, 2009; Chandra *et al.*, 2010; Mueller *et al.*, 2010; Pelusi *et al.*, 2014; Jarin *et al.*, 2017) and are also similar to the hematocrit changes in hypogonadal men receiving testosterone treatment (Saad *et al.*, 2011). Hypogonadal men often present with markedly decreased serum hematocrit, which increases significantly 3 months after the initiation of testosterone treatment, with peak levels at 9 to 12 months (Saad *et al.*, 2011).

We report a decrease in serum hematocrit levels in all trans women in our study. This is consistent with results of Roberts *et al.* (2014), who reported serum hematocrit levels similar to cisgender women in trans women on gender-affirming hormonal treatment, and of Schlatterer *et al.* (1998), who reported a decrease in serum hematocrit levels in trans women receiving estrogens with cyproterone acetate. Furthermore, the time course of the decrease in hematocrit in the trans women in our study is comparable to the hematocrit changes in cisgender men undergoing androgen deprivation treatment for prostate cancer, with the most pronounced decrease occurring in the first 3 months (Strum *et al.*, 1997; D'Amico *et al.*, 2002). Interestingly, a recently published retrospective observational study (Jarin *et al.*, 2017) has not found a change in hematocrit in trans women receiving gender-affirming hormonal treatment. We hypothesize that this can be explained by a less optimal lowering of endogenous testosterone levels in these patients. The authors reported that testosterone levels had decreased to 256 ng/dL at 3 months in trans women receiving estrogens with or without spironolactone, but nearly all subjects maintained serum

Table 2 Comparative analysis on prospective serum hematocrit, testosterone, and estradiol levels

Months	Baseline	3	6	9	12	18	24	36	*p*-value of linear regression
Trans men									
Serum hematocrit levels (95% CI)	41.0 [39.0–42.6] (40.7–41.5)	43.8 [43.8–46.0] (43.1–44.1%)	44.1 [42.1–45.9] (43.2–44.6%)	45.0 [43.7–46.9] (44.3–45.6%)	46.0 [44.0–47.0] (44.8–45.9%)	45.0 [42.0–46.9] (43.1–44.9%)	45.4 [43.3–47.8] (43.4–46.0%)	44.8 [43.0–47.9] (43.7–46.6%)	<0.001*
Compared to previous visit		$p < 0.001$*	$p < 0.001$*	$p < 0.001$*	$p < 0.001$*	$p < 0.001$*	$p < 0.001$*	$p < 0.001$*	
Serum testosterone levels (ng/dL)	37.0 [25.5–55.6]	491.0 [289.5–655.3]	526.0 [425.5–676.0]	630.0 [460.0–707.8]	841.0 [551.0–1131.0]	533.7 [451.3–733.3]	625.4 [468.4–875.0]	628.3 [410.3–892.6]	<0.001*
Compared to previous visit		$p < 0.001$*	$p = 0.988$	$p = 0.166$	$p < 0.001$*	$p < 0.001$*	$p = 0.015$*	$p = 0.725$	
Serum estradiol levels (pg/mL)	37.2 [23.7–85.3]	35.7 [26.3–48.1]	37.1 [27.6–49.8]	35.15 [27.0–44.9]	45.5 [31.9–60.2]	28.7 [25.0–36.4]	32.8 [25.0–43.8]	44.6 [28.0–61.1]	<0.001*
Compared to previous visit		$p = 0.011$*	$p = 0.391$	$p = 0.890$	$p = 0.062$	$p < 0.001$*	$p = 0.016$*	$p = 0.025$*	
Trans women									
Serum hematocrit levels (95% CI)	45.1% [42.7–47.5] (44.9–45.5)	41.0 [39.9–43] (40.9–41.7%)	41.1 [39.6–42.8] (40.9–41.7%)	41.0 [39.6–42.2] (40.5–41.2%)	41.7 [40.0–42.9] (41.1–41.8%)	40.4 [39.0–42.0] (40.0–40.8%)	40.7 [39.2–42.4] (40.5–41.4%)	41.1 [39.4–42.3] (40.3–41.5%)	<0.001*
Compared to previous visit		$p < 0.001$	$p = 0.002$	$p = 0.500$	$p = 0.099$	$p = 0.029$	$p = 0.126$	$p = 0.995$	
Serum testosterone levels (ng/dL)	500.8 [354.3–620.0]	20.3 [14.5–29.0]	16.9 [10–26.3]	15.2 [10.0–21.3]	20.3 [14.5–26.8]	13.9 [10.0–20.9]	17.7 [10.2–24.0]	13.2 [10.0–21.6]	<0.001*
Compared to previous visit		$p < 0.001$*	$p = 0.951$	$p = 0.084$	$p = 0.163$	$p = 0.044$*	$p = 0.127$	$p = 0.579$	
Serum estradiol levels (pg/mL)	26.7 [22.4–33.1]	59.1 [33.8–91.4]	71.7 [41.0–97.9]	76.5 [54.2–90.4]	63.0 [41.7–99.7]	64.5 [45.1–86..9]	61.8 [37.9–89.6]	63.1 [44.8–109.0]	<0.001*
Compared to previous visit		$p < 0.001$*	$p = 0.071$	$p = 0.799$	$p = 0.736$	$p = 0.733$	$p = 0.661$	$p = 0.692$	

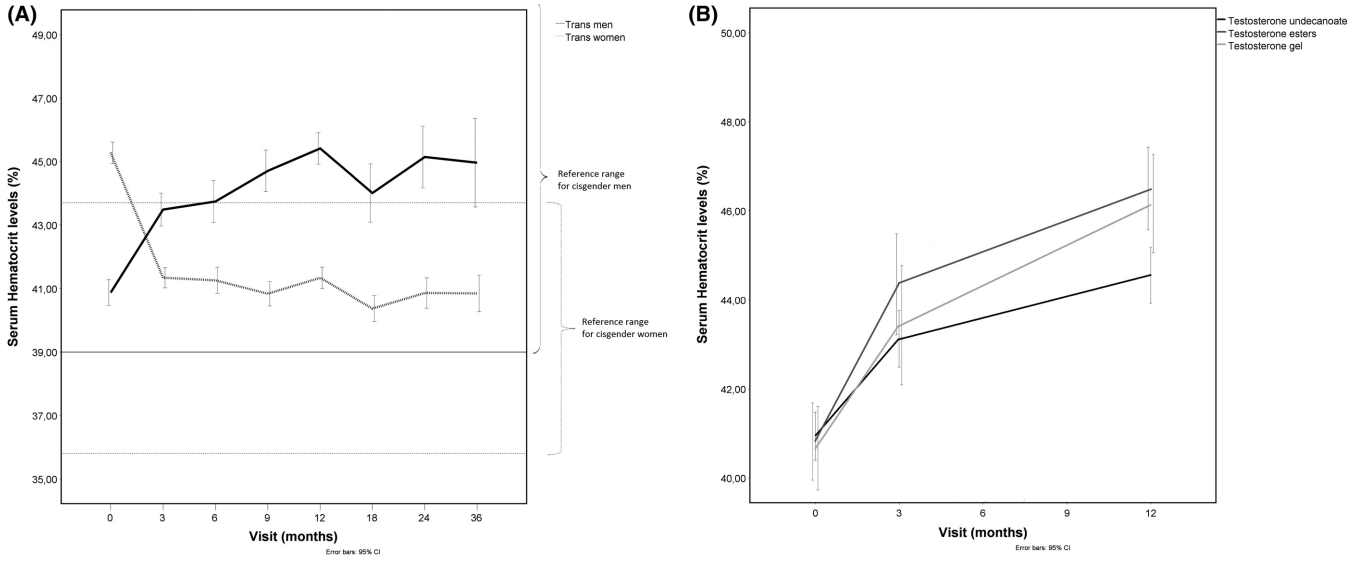
Prospective analysis of serum hematocrit, testosterone, and estradiol levels, for trans men and trans women, during each visit. For values that are not normally distributed, median values and IQR [P25 and P75] are shown. Serum levels of hematocrit, testosterone, and estradiol were compared to the previous visit using Wilcoxon signed rank test. Statistically significant differences are marked with * ($p < 0.05$).

testosterone levels above female reference ranges. In our cohort of trans women, we documented effective androgen deprivation through the administration of 50 mg cyproterone acetate once daily. Our observation that trans women presenting with higher serum hematocrit levels had relatively higher serum testosterone levels further supports the predominant role of androgen deprivation in the development of lowered hematocrit levels. Furthermore, Tack *et al.* (2017) have recently shown that monotherapy with 50 mg cyproterone acetate once daily is sufficient to induce a 3.1 Hct % drop after 6 months in a cohort of 27 adolescent trans girls. The subsequent administration of estrogens was not associated with any further significant changes in hematocrit levels in these patients.

Also from a physiological point of view, the observed hematocrit changes are thought to be primarily driven by changes in testosterone. The first observation that androgens exert an erythrogenic effect dates back from 1962 (Kennedy, 1962), and different possible mechanisms to explain this effect have been proposed. Bachman *et al.* (2014) proposed a multifactorial mechanism in which testosterone induces erythropoiesis through a change in the physiological erythropoietin set point for a given hemoglobin level, combined with an increase in iron use for erythropoiesis. The latter effect is reflected by suppressed hepcidin levels (increasing iron absorption, iron systemic transport, and erythropoiesis) (Bachman *et al.*, 2010) and increased soluble transferrin receptor levels (reflecting increased erythroid activity, plasma iron turnover, and erythroid transferrin uptake) (Bachman *et al.*, 2014). Apart from these indirect effects, evidence also exists for a direct effect on hematopoietic stem cells through stimulation of ERα receptors by estrogens derived from the local aromatization of testosterone (Calado *et al.*, 2009).

Different authors have pointed out the need for specific reference ranges for laboratory test results in trans persons (Roberts *et al.*, 2014; Colizzi *et al.*, 2015; Kandhro, 2016). Although it has not been established yet whether totally new reference ranges should be developed or whether the reference ranges of the perceived gender should be applied, to avoid major errors, it seems important that there should be at least some guidance for clinicians about what to expect in their patients. Of course, one should bear in mind that the proposed reference intervals do not necessarily correlate with the same clinical endpoints as in cisgender persons because the bodies of trans persons go through a broad range of changes during the transition period. For example, apart from changes in hematocrit, gender-affirming hormonal treatment also has an impact on a range of other factors that are associated with cardiovascular health, such as changes in body fat composition (Elbers *et al.*, 1997), cholesterol levels, and glucose metabolism (Elbers *et al.*, 2003). As these changes primarily occur during the first year of hormonal treatment, this period should receive particular attention (Colizzi *et al.*, 2015). As shown in Fig. 2a, our results show that serum hematocrit levels can be found in the reference range of the perceived gender as from 3 months after the initiation of gender-affirming hormonal treatment. Therefore, we suggest consulting the reference range for men in trans men after the initiation of testosterone treatment and the reference range for women in trans women in whom effective androgen deprivation has been established (either through anti-androgens or after orchiectomy).

Figure 2 Prospective analysis of serum hematocrit levels in trans men and trans women (a) and prospective analysis of serum hematocrit levels in trans men, based on mode of testosterone administration. (b) Line graphs for the evolution of median serum hematocrit levels in trans men and trans women on gender-affirming hormonal treatment (a) and trans men on different types of testosterone (b), with 95% confidence intervals. Tables underneath the graphs describe the number of persons included in each group at each given time point. (a) Reference ranges for serum hematocrit levels in cisgender men (39.0–49.7%) and women (35.8–43.7%), according to the Ghent University Hospital laboratory, are displayed with accolades. (b) Only visits with $n \geq 5$ for all groups are shown on the graph.



	M0	M3	M6	M9	M12	M18	M24	M36
Trans men (n)	192	184	96	88	146	82	67	20
Trans women (n)	239	208	133	125	186	115	90	58

	M0	M3	M6	M9	M12	M18	M24	M36
Testosterone undecanoate (n)	109	103	88	87	79	82	67	20
Testosterone esters (n)	44	43	5	1	34	0	0	0
Testosterone gel (n)	39	38	0	0	33	0	0	0

Table 3 Comparative analysis on serum hematocrit levels in trans men on different testosterone agents

Months	TU	TE	TG	*p*-value for difference between three groups
0	40.9 [39.25–42.4] (30.2–49.0)	41.0 [39.0–43.0] (35.0–47.6)	40.0 [39.0–42.8] (34.0–47.0)	$p = 0.886$
3	43.5 [41.4–45.3] (32.2–50.3)	44.0 [42.0–47.0] (37.0–51.0)	43.0 [41.0–46.0] (31.0–50.0)	$p = 0.154$
6	44.0 [42.3–45.8] (31.8–50.0)	48.0 [42.0–50.5] (41.0–52.0)	36 [36.0–38.0] (36.0–40.0) °	$p = 0.004$*
9	45.0 [43.6–46.8] (33.0–50.3)	No data	No data	
12	45.1 [43.1–46.4] (36.5–49.7)	46.0 [44.75–48.25] (42.0–52.0)	46.5 [44.0–48.0] (37.0–52.0)	$p < 0.001$*

Comparative analysis on serum hematocrit levels in trans men taking testosterone undecanoate (TU), testosterone esters (TE) and testosterone gel (TG), during the first year of gender-affirming hormonal treatment. For values that are not normally distributed, median values and IQR [P25 and P75] are shown. Groups were compared using the Kruskal–Wallis test. Statistically significant differences are marked with * ($p < 0.05$). ° $n = 3$ for the group taking testosterone gel at 6 months.

As in hypogonadal men on testosterone treatment, the induction of erythrocytosis in trans men on testosterone treatment is of concern, because of the potentially increased risk for venous thromboembolism, cerebrovascular accidents, and myocardial infarction. Absolute erythrocytosis is defined as a red cell mass of >125% of that predicted for body mass and gender (Keohane *et al.*, 2013). The red cell mass can be measured through a highly specialized nuclear medicine test, but this is rarely used in clinical practice. Instead, absolute erythrocytosis can be suspected when serum hematocrit is raised (but this does not discriminate from apparent erythrocytosis, which is normal red cell mass with reduced plasma volume). An often-cited cutoff value in men is hematocrit >52% in men (Keohane *et al.*, 2013). Because hematocrit >50% as a cutoff has been used in some testosterone studies (Middleton *et al.*, 2015; Pastuszak *et al.*, 2015), we have decided to calculate rates using both cutoffs in our study (making comparison possible). Traditionally, the increased blood viscosity resulting from increased red blood cell mass has been associated with thrombotic complications, and although primary polycythemia is associated with an increased thrombotic risk (McMullin *et al.*, 2016), a causal effect has not been proven for secondary polycythemia. A 2014 review on the risk for arterial or venous thrombosis in patients with polycythemia secondary to cardiac or pulmonary disease, smoking, or idiopathic causes concluded that 'there is no definite evidence that secondary polycythemia increases the risk of thromboembolism' (Bhatt, 2014). Recently, there has been an increased interest in possible cardiovascular and venous thromboembolism risks associated with testosterone treatment in hypogonadal men, without associating these with erythrocytosis per se. A recent meta-analysis

by Corona *et al.* (2017) concluded that 'testosterone therapy is not associated with an increased risk for venous thromboembolism' and 'available data do not support an increased cardiovascular risk related to testosterone therapy'. In trans men on testosterone treatment, prior retrospective cohort studies have shown no increased risk for venous or arterial thrombosis (van Kesteren *et al.*, 1997; Schlatterer *et al.*, 1998; De Cuypere *et al.*, 2005; Ott *et al.*, 2010; Wierckx *et al.*, 2012, 2013, 2014). In our study cohort, we have observed low erythrocytosis rates in trans men on testosterone treatment, with a maximum measured hematocrit of 54.0%, and none of the trans men experienced any thromboembolic events during follow-up.

Comparative studies in hypogonadal men have shown that the rate of erythrocytosis is higher in men receiving short-acting intramuscular injections, compared to transdermal or subcutaneous routes (Dobs *et al.*, 1999; Pastuszak *et al.*, 2015). An explanation might be found in the fact that short-acting intramuscular injections cause a rapid supraphysiological peak shortly after injection, decreasing to low levels when the next injection is near, whereas transdermal systems and pellets result in more stable serum concentrations (Ohlander *et al.*, 2017). Administration of testosterone undecanoate, an oil vehicle-based formulation which is injected intramuscularly every 12 weeks, results in more stable serum concentrations than intramuscular injections with testosterone esters (Schubert *et al.*, 2004). A prospective observational study by Middleton *et al.* (2015) has examined erythrocytosis rates in a population of 347 patients (both hypogonadal males and trans men) receiving a total of 3022 injections of testosterone undecanoate over 3.5 years. Only 25 patients (7%) developed hematocrit levels >50% (Middleton *et al.*, 2015), whereas in the study by Pastuszak *et al.* (2015), 66.7% of hypogonadal males receiving testosterone esters developed hematocrit levels >50%. In our study cohort, trans men receiving testosterone esters had a larger increase in serum hematocrit levels during the 3-year follow-up period, compared to trans men receiving testosterone undecanoate (Δ 0.8 Hct %). In addition, trans men on testosterone undecanoate exhibited lower erythrocytosis rates (9.2% were >50 Hct %, and 0 were >52 Hct %), compared to trans men on testosterone esters (15.9% were >50 Hct %, and 6.8% were >52 Hct %) or gel (12.8% were >50 Hct %, and 2.6% were >52 Hct %). We were not able to correlate this finding with differences in testosterone levels, which might be explained by the fact that pharmacokinetic profiles of these formulations mostly differ with respect to peak concentrations and overall stability of serum concentrations (Ohlander *et al.*, 2017), parameters which were not measured in our study.

'Endocrine Treatment of Gender-Dysphoric/Gender-Incongruent Persons: An Endocrine Society Clinical Practice Guideline' (Hembree *et al.*, 2017) recommends determining hematocrit in trans men on testosterone treatment at baseline, every 3 months for the first year and then one to two times a year thereafter. This guideline warns about the induction of erythrocytosis in trans men and discourages the initiation of testosterone treatment if baseline hematocrit exceeds 50%. In addition, in the Endocrine Society Clinical Practice Guideline for Testosterone Therapy in Men with Androgen Deficiency Syndromes (Bhasin *et al.*, 2010), it is suggested to interrupt testosterone treatment if hematocrit increases above 54%, until hematocrit has decreased to safe levels, after which treatment can be reinitiated at a reduced dose. We suggest to also apply this advice when treating trans men with testosterone. In our opinion, switching from testosterone esters to testosterone undecanoate seems a valid alternative option in selected cases. In this way, interruption of testosterone treatment (and the associated psychological burden for the patient) can be avoided.

Our study results may have been affected by several limitations. In some patients in the Amsterdam cohort, hematocrit was not assessed at all time points. In addition, follow-up in

Table 4 Number of trans men with elevated serum hematocrit levels (>50.0% and >52.0%, respectively), by mode of testosterone administration

Number of trans men with elevated serum hematocrit levels (≥ 50.0%)

Months	0	3	6	9	12	18	24	36	Total measurements on gender-affirming treatment
Number/total (%)	0/189	8/184 (4.3%)	2/96 (2.1%)	2/88 (2.3%)	10/146 (6.8%)	0/82 (6.8%)	7/67 (10.4%)	1/20 (5.0%)	30/683 (4.4%)
Type of gender-affirming treatment									
Testosterone Undecanoate	0/106	1/103 (1.0%)	1/88 (1.1%)	2/87 (2.3%)	0/79	0/82	7/67 (10.4%)	1/20 (5.0%)	12/526 (2.2%)
Testosterone Esters	0/44	5/43 (11.6%)	1/5 (20.0%)	No data	5/34 (14.7%)	No data	No data	No data	11/82 (13.4%)
Testosterone gel	0/36	2/35 (5.3%)	0/3	0/1	5/32 (15.6%)	No data	No data	No data	7/71 (9.9%)

Number of trans men with elevated serum hematocrit levels (>= 52.0%)

	0	3	6	9	12	18	24	36	Total
Number/total (%)	0/189	0/184	1/96 (1.0%)	0/88	4/146 (2.7%)	0/82	0/67	0/20	5/683 (0.7%)
Type of gender-affirming treatment									
Testosterone Undecanoate	0/106	0/103	0/88	0/87	0/79	0/82	0/67	0/20	0/526
Testosterone Esters	0/44	0/43	1/5 (20.0%)	No data	3/34 (8.8%)	No data	No data	No data	4/82 (4.9%)
Testosterone gel	0/36	0/35	0/3	0/1	1/32 (3.1%)	No data	No data	No data	1/71 (1.4%)

Number of trans men with serum hematocrit levels above 50% and 52%, respectively. Data are shown for the total group of trans men and for each testosterone agent (testosterone undecanoate (TU), testosterone esters (TE) and testosterone gel (TG). Groups were compared using chi-square test. Statistically significant differences are marked with * ($p < 0.05$).

Amsterdam only took place during the first year of gender-affirming hormonal treatment, leading to a decrease in sample size and power in the analyses of the 18th, 24th, and 36th months. As a further consequence of this, along with the fact that trans men included in the ENIGI study in Ghent exclusively received testosterone undecanoate injections, we were not able to prospectively evaluate serum hematocrit levels of trans men on other testosterone agents after more than one year of treatment. In addition, blood samples were obtained at fixed time points during the follow-up period, independent of the time interval to the last administration. This may have led to fluctuations in measured serum testosterone and estradiol levels.

Despite these limitations, this study has a number of strengths. To our knowledge, this is the largest prospective study to date in which serum hematocrit levels in both trans men and trans women were evaluated. Our study cohorts are well defined and participants adhered to a strict treatment regimen. In addition, this is the first large study that directly compared the effects of intramuscular testosterone esters vs. intramuscular testosterone undecanoate on hematocrit levels and erythrocytosis rates.

CONCLUSIONS AND SUGGESTIONS FOR CLINICAL PRACTICE

1 Serum hematocrit levels can be found in the reference range of the perceived gender as from 3 months after the initiation of gender-affirming hormonal treatment. We suggest consulting the reference range for men in trans men after the initiation of testosterone treatment and the reference range for women in trans women in whom effective androgen deprivation has been established.
2 As we describe low erythrocytosis rates in trans men on testosterone treatment, with a maximum measured hematocrit level of 54.0%, and as none of the trans men in our study cohort experienced a thromboembolic event during follow-up, we have no reasons to assume that the observed mild increase in serum hematocrit levels is associated with an increased thrombotic risk on short term.
3 Trans men on testosterone undecanoate exhibit lower erythrocytosis rates compared to trans men on testosterone esters or gel. Changing the treatment to testosterone undecanoate seems a valid option if the hormone prescribing physician and/or the patient are concerned about elevated serum hematocrit levels. This may prevent unnecessary interruptions in hormonal treatment.

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DECLARATION OF INTEREST

The authors report no conflict of interests in this work.

AUTHORS' CONTRIBUTIONS

Defreyne J and Vantomme B are first authors, conceived and designed the study, analyzed and interpreted the data, and drafted the article; Vantomme B revised the article critically for important intellectual content. Van Caenegem E, Wierckx K, De Blok C, Klaver M, Nota N, Van Dijk D, and Wiepjes C are co-authors, analyzed and interpreted the data, and drafted the article. Den Heijer M and T'Sjoen G are principal investigators, conceived and designed the study, and revised the article critically for important intellectual content. All authors approved the final version to be published.

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