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Review

Laboratory Monitoring in Transgender and Gender-Diverse Individuals

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BACKGROUND: Increasing numbers of transgender and gender-diverse individuals are seeking initiation of gender-affirming hormone therapy. This aligns an individual's physical characteristics with their gender identity and improves psychological outcomes. Physical changes, including changes to muscle mass and body fat redistribution, can alter sex-specific laboratory reference ranges.

CONTENT: We review the impact of gender-affirming hormone therapy on laboratory parameters with sex-specific reference ranges, with a focus on hemoglobin/hematocrit, renal function, cardiac biomarkers, and prostate-specific antigen.

SUMMARY: Gender-affirming hormone therapy results in changes in laboratory parameters with sex-specific reference ranges. For individuals established on gender-affirming hormone therapy, reference ranges that align with an individual's gender identity should be used for hemoglobin/hematocrit, serum creatinine, and high-sensitivity cardiac troponin and N-terminal brain natriuretic peptide. Clinicians should interpret these biomarkers according to the reference range that aligns with one's affirmed gender.

Introduction

With an exponential increase in the number of transgender and gender-diverse (trans) individuals (including those with binary and nonbinary identities) seeking gender-affirming hormone therapy (GAHT) worldwide over the last decade, it is pertinent that clinicians, pathologists, and laboratory scientists are aware of the impact of GAHT on biochemical tests. GAHT (masculinizing or feminizing) is desired by many trans people to induce physical characteristics to align with an individual's gender identity. Commencement of GAHT is a carefully considered decision, balancing the benefits on mental health outcomes with potential risks and adverse effects (1). Importantly, GAHT has been shown to reduce gender dysphoria and improve mental health and quality of life (2–4). Standard GAHT formulations and doses are shown in Table 1.

Trans people recorded male at birth who commence feminizing hormone therapy are typically treated with estradiol and an antiandrogen, such as cyproterone acetate, spironolactone, or gonadotropin releasing hormone (GnRH) analogs. This induces development of physical changes that aligns with one's gender identity, including changes in body composition manifest as increases in fat mass by 20%–30% and changes to body fat redistribution (onset 3–6 months, maximal change 2–3 years), decreases in muscle mass by 3%–5% over the first 12 months (onset 3–6 months, maximal change 1–2 years), as well as breast development, skin softening, and decreased sexual function (7–10).

Trans people recorded female at birth who commence masculinizing hormone therapy are treated with testosterone formulations typically administered to hypogonadal cisgender men. This results in increases in muscle mass by 10% (onset 6–12 months, maximal change 2–5 years), decreases in fat mass by 10%, as well as body fat redistribution (onset 1–6 months, maximal change 2–5 years), voice deepening, development of facial and body hair, and menstrual cessation (7–10). Notably, short-acting intramuscular testosterone esters have been associated with greater changes to lean mass and body fat (10) and hematocrit (11), compared to long-acting intramuscular undecanoate or transdermal testosterone.

In the general population, differences in sex steroid concentrations and/or body size impact many laboratory parameters including hemoglobin/hematocrit, creatinine, and cardiac troponin, which have sex-specific reference ranges. However, the influence of GAHT on these parameters has been poorly defined. We aim to provide a summary of the impact of GAHT on parameters with sex-specific reference ranges, including hemoglobin/hematocrit, creatinine, and cardiac troponin.

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Table 1. Hormone regimens in transgender and gender-diverse individuals.[a]

Hormone regimen	Dose
Estradiol	
Oral estradiol valerate/ hemihydrate	2–6 mg/day
Transdermal estradiol patch	0.025–0.2 mg/day
Transdermal estradiol gel	0.75–3 mg/day
Parenteral estradiol valerate or cypionate	2–10 mg IM or SC/week
Antiandrogen	
Spironolactone	100–300 mg/day
Cyproterone acetate	10–25 mg/day (5, 6)
GnRH agonist	3.75 mg SC monthly or 11.25 mg SC 3-monthly
Testosterone	
Parenteral testosterone enanthate/cypionate	100–250 mg IM or SC fortnightly
Parenteral testosterone undecanoate	1000 mg every 12 weeks
Transdermal testosterone gel	50–100 mg/day
Transdermal testosterone patch	2.5–7.5 mg/day

Abbreviations: IM, intramuscular; SC, subcutaneous.
[a]Adapted from Hembree WC, Cohen-Kettenis PT, Gooren L, Hannema SE, Meyer WJ, Murad MH, et al. Endocrine treatment of gender-dysphoric/gender-incongruent persons: An endocrine society clinical practice guideline. *The Journal of Clinical Endocrinology and Metabolism* 2017;102(11):3869–903, (7) by permission of the Endocrine Society and Oxford University Press.

Sex Steroid Concentrations

FEMINIZING HORMONE THERAPY

Consensus guidelines give recommendations for sex steroid concentrations in trans individuals undergoing GAHT, generally within the range of the affirmed gender. However, these ranges are based on expert opinion, and differences exist internationally. In trans individuals undergoing feminizing hormone therapy, Endocrine Society guidelines recommend targeting serum estradiol concentration 100–200 pg/mL (367–734 pmol/L) and serum testosterone <50 ng/dL (<1.7 nmol/L) (7). However, clinicians in the Amsterdam Cohort of Gender dysphoria target serum estradiol concentrations >54.4 pg/mL (200 pmol/L) for bone health (12), and Australian guidelines recommend targeting serum estradiol 68.1–163.4 pg/mL (250–600 pmol/L) and total testosterone <57.7 ng/dL (<2 nmol/L) based on local cross-sectional data (1). No study has evaluated optimal serum sex steroid concentrations in trans individuals undergoing feminizing hormone therapy and it is not clear if monitoring sex steroid concentrations is clinically useful (13). Current data has not found a relationship between serum estradiol concentration and breast development (14) or body fat redistribution (10). However, higher serum estradiol concentrations are associated with higher bone density (15). It is important to note that estradiol doses are often gradually uptitrated, which may influence the degree and rate of body composition changes with resultant effects on sex-specific laboratory parameters.

Serum estradiol concentrations achieved with various estradiol regimens have been assessed in several cohorts, and it should be noted that serum estradiol concentration varies depending on estradiol formulation. An Australian cross-sectional study revealed a median serum estradiol concentration of 89 pg/mL (328 pmol/L) with oral estradiol valerate (16), whereas a cohort in the United States reported serum estradiol concentrations of 366 pg/mL (1343 pmol/L) with intramuscular estradiol, 102 pg/mL (374 pmol/L) with oral estradiol, and 70.8 pg/mL (259 pmol/L) with transdermal estradiol (17). Injectable estradiol is more likely to result in higher serum estradiol concentrations (17, 18). Timing of monitoring of serum estradiol concentration is another important consideration, particularly with sublingual estradiol that can cause significant peaks post-dose (19).

Using a cohort of 93 trans individuals from the United States, a cross-sectional study reported a reference interval for serum estradiol measured via LC-MS/MS of 20.7–505 pg/mL (76–1853 pmol/L) (20). Within this cohort, it was also reported that estradiol concentrations measured via immunoassay were lower than those measured via LC-MS (21). This is an important consideration for practitioners, who frequently dose-adjust based on serum estradiol concentrations. Some of the discrepancy between immunoassay and LC-MS could be attributed to differences in serum estrone concentration between estradiol formulations. Estrone is an estradiol metabolite with weaker affinity at the estrogen receptor. It is known that oral and sublingual estradiol produce higher serum estrone concentrations than transdermal estradiol (22, 23). Importantly, serum estrone concentration does not appear to influence feminization (23).

Consensus guidelines give recommendations for testosterone suppression within the cisgender female

reference range (7, 8). However, peripheral androgen receptor antagonists such as spironolactone or bicalutamide may not necessarily lower serum testosterone concentrations within this range (24, 25).

MASCULINIZING HORMONE THERAPY

For trans individuals undergoing masculinizing hormone therapy, consensus guidelines recommend targeting serum testosterone concentrations within the cisgender male range [320–1000 ng/dL (11.1–34.7 nmol/L)] (7). A serum total testosterone reference interval of 199–1149 ng/dL (6.9–39.9 nmol/L) for those on stable masculinizing hormone therapy regimens has been established in a cohort of 82 trans people treated with testosterone from the United States (26).

Intramuscular testosterone has been associated with higher serum testosterone concentrations than transdermal testosterone in some analyses (17). Clinically, higher serum testosterone concentrations have been associated with a greater decrease in body fat (10) and menstrual cessation (27) but can increase the risk of polycythemia. There are also modest decreases in serum estradiol concentration with testosterone therapy, although this does not alter masculinization and there is no guidance regarding the utility of monitoring (28).

Some trans people, including nonbinary people, may use low-dose testosterone to develop more gradual physical effects, or a lesser degree of physical effects, which can result in serum testosterone concentrations between the cisgender female and male reference range (29–31).

Hemoglobin/Hematocrit

The effect of GAHT on red blood cell parameters is well-documented. Androgens are known to stimulate erythropoiesis (32), and resultant changes in red blood cell parameters are seen in the trans population. In trans people undergoing feminizing hormone therapy for at least 6 months, longitudinal studies have demonstrated that hemoglobin/hematocrit decrease to the cisgender female reference range (11, 33–35). In trans people undergoing masculinizing hormone therapy for at least 6 months, hemoglobin/hematocrit increase to the cisgender male reference range (11, 34–36). Serum hematocrit aligns with the affirmed gender after 3 months of GAHT (11).

Studies evaluating the influence of GAHT on hemoglobin/hematocrit are shown in Table 2.

CLINICAL IMPLICATIONS

In trans people undergoing masculinizing hormone therapy, there is a well-documented risk of polycythemia (35, 49–52). This risk appears higher with intramuscular compared to transdermal testosterone formulations (49, 50). Notably, population-based studies have demonstrated associations between higher hematocrit and a higher risk of cardiovascular disease in the general population (53, 54). However, it should be noted that use of different reference ranges for hemoglobin and hematocrit may mean that effects on cardiovascular risk remain unclear. Based on the available data, the cisgender female reference range should be used for people taking feminizing GAHT, whereas the cisgender male reference range should be used for people taking masculinizing GAHT. Smoking cessation should also be recommended, if relevant, given that it may additionally increase hematocrit.

Renal Function

The most commonly used marker of renal function is estimated glomerular filtration rate (eGFR), which is calculated based on an individual's serum creatinine concentration, age, and sex (55). At the same serum creatinine concentration, people recorded male at birth have a higher eGFR than those recorded female at birth because the eGFR equations assume that people recorded male at birth typically have a higher muscle mass contributing to the serum creatinine concentration independent of renal function. Given significant changes to body composition following initiation of GAHT (lean body mass increases with masculinizing and decreases with feminizing GAHT) (9), it is imperative to interpret serum creatinine and eGFR based on duration of GAHT.

LONGITUDINAL CHANGES IN RENAL FUNCTION IN TRANS PEOPLE

Longitudinal studies have documented increases in serum creatinine in trans people undergoing masculinizing GAHT (48, 56, 57), and decreases in serum creatinine for those undergoing feminizing GAHT (48, 56, 57). However, there is variability between studies and many studies are limited by small sample sizes and variability in GAHT regimens. Increases in serum creatinine are evident from 3 to 6 months following initiation of testosterone therapy, but there is variability between studies in people initiating estradiol; some demonstrate significant decreases in creatinine compared to baseline (36, 56) but others do not (33, 34) at 3–6 months. By 12 months, a significant decrease in creatinine has been reported in several studies (34, 39, 48, 56, 58).

A systematic review and meta-analysis of the influence of GAHT regimens on renal function included 488 transgender men and 593 transgender women (59). Twelve months after initiation of GAHT, serum creatinine increased [+0.15 mg/dL (95% CI, 0.00 to 0.29)]

Table 2. Summary of studies investigating hemoglobin/hematocrit in transgender individuals.

Reference	Country	Study type	Duration	Treatment regimen (number); sex steroid concentrations	Results
Schlatterer et al., 1998 (37)	Germany	Retrospective, cohort No control group	Not reported	E2 ($n = 46$) T ($n = 42$) Sex steroid concentrations not reported	Hemoglobin decreased in trans individuals treated with estradiol Hemoglobin increased in trans individuals treated with testosterone
Jacobeit et al., 2009 (38)	Germany	Cohort No control group	36 months	T ($n = 17$) Testosterone concentration confirmed in male reference range	**Trans individuals treated with testosterone:** Hemoglobin, g/dL: Baseline vs 36 months, mean (SD): 13.6 (1.2) vs 16.0 (1.5) Hematocrit, %: Baseline vs 36 months, mean (SD): 41 (4) vs 46 (4)
Wierckx et al., 2014 (39)	Belgium, Norway	Prospective, cohort No control group	12 months	E2 ($n = 53$) T ($n = 53$) Sex steroid concentrations confirmed within target ranges	**Trans individuals treated with estradiol:** Hematocrit, %: Baseline vs 12 months, mean (SD): Oral: 45.2 (2.5) vs 42.0 (5.7), $P = 0.003$ Transdermal: 45.5 (1.7) vs 42.0 (2.3), $P < 0.001$ **Trans individuals treated with testosterone:** Hematocrit, %: Baseline vs 12 months, mean (SD): 40.8 (2.9) vs 45.8 (3.0), $P < 0.001$
Pelusi et al., 2014 (40)	Italy	Prospective, cohort No control group	54 weeks	T ($n = 45$) Testosterone concentration confirmed within target range	**Trans individuals treated with testosterone:** Hemoglobin, g/dL: Baseline vs 30 weeks vs 54 weeks: Testosterone propionate/testosterone enanthate, mean (95% CI): 12.6 (12.0–13.2) vs 13.4 (12.5–14.3) vs 14.3 (13.5–15.2), $P < 0.0005$ Testosterone gel: 13.5 (12.9–14.0) vs 13.8 (12.98–14.7) vs 14.1 (13.2–14.9), $P < 0.0005$

Continued

Table 2. (continued)

Reference	Country	Study type	Duration	Treatment regimen (number); sex steroid concentrations	Results
					Testosterone undecanoate: 13.3 (12.7–13.8) vs 14.1 (13.3–14.9) vs 14.6 (13.8–15.4), $P < 0.0005$ Hematocrit, %: Baseline vs 30 weeks vs 54 weeks: Testosterone propionate/testosterone enanthate, mean (95% CI): 38.4 (36.8–39.9) vs 41.2 (38.9–43.4) vs 43.1 (40.9–45.2), $P < 0.0005$ Testosterone gel: 40.6 (39.1–42.2) vs 41.5 (39.4–43.6) vs 42.6 (40.5–45.3), $P < 0.0005$ Testosterone undecanoate: 39.1 (37.7–40.5) vs 42.2 (40.2–44.1) vs 43.4 (41.5–45.3), $P < 0.0005$
Roberts et al., 2014 (41)	United States	Cross-sectional CW and CM controls	N/A>6 months E2 (median 4 years)	E2 ($n = 55$) CW ($n = 20$) CM ($n = 20$) Sex steroid concentrations not reported	**Trans individuals treated with estradiol:** Hemoglobin, g/dL: Trans people vs CM vs CW (2.5th–97.5th percentile): 12.6–14.7 vs 13.3–16.2 vs 11.9–14.6 Hematocrit, %: Trans people vs CM vs CW (2.5th–97.5th percentile): 34.6–43.7 vs 38.4–45.7 vs 34.4–41.9
Gava et al., 2016 (42)	Italy	Retrospective, cohort No control group	12 months	E2 ($n = 40$) Sex steroid concentrations confirmed within target ranges	**Trans individuals treated with estradiol:** Hematocrit, %: Baseline vs 12 months, mean (SD): Estradiol and cyproterone acetate: 43.5 (4.5) vs 40.2 (3.1), $P < 0.05$ Estradiol and leuprolide acetate: 44.8 (2.3) vs 40.5 (2.3), $P < 0.05$
Fernandez et al., 2016 (36)	United States	Retrospective,	6–18 months	E2 ($n = 33$) T ($n = 19$)	**Trans individuals treated with testosterone:** Hemoglobin, mean (SE), g/dL:

Continued

Table 2. (continued)

Reference	Country	Study type	Duration	Treatment regimen (number); sex steroid concentrations	Results
		cohort No control group		Sex steroid concentrations not reported	Baseline vs 3–6 months vs 6–18 months: 13.4 (0.3) vs 14.2 (0.4) vs 15.2 (0.4), $P < 0.01$ vs baseline Hematocrit, mean (SE), %: Baseline vs 3–6 months vs 6–18 months: 39.8 (0.8) vs 43.4 (1.1) vs 45.9 (1.2), $P < 0.01$ vs baseline
Gava et al., 2018 (43)	Italy	Retrospective, cohort No control group	5 years	T ($n = 50$) Serum testosterone concentration confirmed within target range	**Trans individuals treated with testosterone:** Hemoglobin, mean (SD), g/dL: Baseline vs 5 years: Testosterone undecanoate: 13.3 (0.9) vs 15.2 (1.3), $P < 0.05$ Testosterone enanthate: 13.1 (1.4) vs 14.1 (3.9), $P < 0.05$ Hematocrit, mean (SD), %: Testosterone undecanoate: 39.7 (2.5) vs 44.9 (3.6), $P < 0.05$ Testosterone enanthate: 39.3 (3.0) vs 44.9 (2.2), $P < 0.05$
Vita et al., 2018 (33)	Italy	Retrospective, cohort No control group	3–6 months	E2 ($n = 21$) T ($n = 11$) Sex steroid concentrations confirmed within target ranges	**Trans individuals treated with estradiol:** Hemoglobin, mean (SD), g/dL: Baseline vs 3–6 months: 15.0 (1.0) vs 13.6 (1.0), $P < 0.01$ Hematocrit, %: Baseline vs 3–6 months: 44.8 (2.9) vs 40.1 (2.6), $P < 0.0001$ **Trans individuals treated with testosterone:** Hemoglobin, mean (SD), g/dL: Baseline vs 3–6 months: 13.5 (0.8) vs 14.7 (1.0), $P < 0.01$

Continued

Table 2. (continued)

Reference	Country	Study type	Duration	Treatment regimen (number); sex steroid concentrations	Results
					Hematocrit, %: Baseline vs 3–6 months: 40.0 (2.9) vs 43.5 (3.1), $P < 0.05$
Defreyne et al., 2018 (11)	Netherlands, Belgium	Prospective, cohort No control group	36 months	E2 ($n = 239$) T ($n = 192$) Sex steroid concentrations confirmed within target ranges	**Trans individuals treated with estradiol:** Hematocrit, median (IQR), %: Baseline vs 3 months vs 12 months: 45.1 [42.7–47.5] vs 41.0 [39.9–43] vs 41.7 [40.0–42.9] **Trans individuals treated with testosterone:** Hematocrit, median (IQR), %: Baseline vs 3 months vs 12 months: 41.0 [39.0–42.6] vs 43.8 [43.8–46.0] vs 46.0 [44.0–47.0]
SoRelle et al., 2019 (44)	United States	Retrospective, cohort No cisgender control group	N/A Groups: No GAHT vs >6 months GAHT	E2 ($n = 133$) vs no E2 ($n = 62$) T ($n = 89$) vs no T ($n = 87$) Sex steroid concentrations confirmed within target ranges	**Trans individuals treated with estradiol:** Hemoglobin, mean (SD), g/dL: Baseline vs post-treatment groups: 15.2 (13.3–17.1) ($n = 73$) vs 13.9 (11.7–16.3) ($n = 105$), $P < 0.0001$ (Cohen's d = 1.14) Hematocrit, mean (SD), %: 44.8 (39.0–50.6) ($n = 73$) vs 41.6 (35.9–48.7) ($n = 105$), $P < 0.0001$ (Cohen's d = 1.00) **Trans individuals treated with testosterone:** Hemoglobin, g/dL: Baseline vs post-treatment groups, mean (SD): 13.1 (11.4–15) ($n = 56$) vs 15.6 (12.4–18.2) ($n = 81$), $P < 0.0001$ (Cohen's d 1.85) Hematocrit, mean (SD), %: Baseline vs post-treatment groups: 39.8 (34.6–45.9) ($n = 56$) vs 46.7 (38.0–53.0), $P < 0.0001$ (Cohen's d 1.98)
	United States	Retrospective,	12 months	E2 ($n = 152$)	**Trans individuals treated with estradiol:**

Continued

Table 2. (continued)

Reference	Country	Study type	Duration	Treatment regimen (number); sex steroid concentrations	Results
Humble et al., 2019 (34)		cohort No control group		T ($n = 150$) Sex steroid concentrations not reported	Hemoglobin, mean (SD), g/dL: Baseline vs 6 months ($n = 23$): 15.0 (1.3) vs 14.1 (1.4), $P < 0.05$ Baseline vs 12 months ($n = 19$): 15.0 (1.4) vs 14.0, $P < 0.001$ Hematocrit, mean (SD), %: Baseline vs 6 months ($n = 23$): 44.2 (3.4) vs 41.0 (4.1), $P < 0.01$ Baseline vs 12 months ($n = 19$): 43.8 (3.6) vs 40.7 (2.4), $P < 0.001$ **Trans individuals treated with testosterone:** Hemoglobin, mean (SD), g/dL: Baseline vs 6 months ($n = 40$): 13.2 (1.3) vs 14.9 (1.5), $P < 0.001$ Baseline vs 12 months ($n = 27$): 13.3 (1.3) vs 15.3 (1.2), $P < 0.001$ Hematocrit, mean (SD), %: Baseline vs 6 months ($n = 37$): 39.6 (3.4) vs 45.3 (3.8), $P < 0.001$ Baseline vs 12 months ($n = 24$): 39.6 (3.7) vs 45.6 (3.5), $P < 0.001$
Greene et al., 2019 (45)	United States	Cross-sectional No control group	N/A	E2 ($n = 93$) T ($n = 79$) Sex steroid concentrations confirmed within target ranges	**Trans individuals treated with estradiol:** Hemoglobin reference range, g/dL: 11.6–15.7 Hematocrit reference range, %: 35.0–47.0 **Trans individuals treated with testosterone:** Hemoglobin reference range, g/dL: 12.8–17.4 Hematocrit reference range: 39–51
Antun et al., 2020 (35)	United States	Cohort study		E2 ($n = 559$)	**Trans individuals treated with estradiol:**

Continued

Table 2. (continued)

Reference	Country	Study type	Duration	Treatment regimen (number); sex steroid concentrations	Results
		Cisgender control groups	Reference range after at least 1-year GAHT	T ($n = 424$) Sex steroid concentrations not reported	Hematocrit reference range, %: 34.1–47.6 **Trans individuals treated with testosterone:** Hematocrit reference range, %: 37.4–52.3
Gava et al., 2020 (46)	Italy	Retrospective, cohort No control group	5 years	E2 ($n = 50$) Sex steroid concentrations confirmed with target ranges	**Trans individuals treated with estradiol:** Hematocrit, (mean SD), %: Baseline vs 12 months: Estradiol and cyproterone acetate: 43.5 (4.5) vs 40.2 (3.1), $P < 0.05$ Estradiol and leuprolide acetate: 44.8 (2.3) vs 40.5 (2.3), $P < 0.05$
Allen et al., 2021 (47)	United States	Retrospective, cohort No control group	60 months	E2 ($n = 126$) T ($n = 91$) Sex steroid concentrations not reported	**Trans individuals treated with estradiol:** Hemoglobin and hematocrit decreased to stable levels by 3 months following E2. All later time points were not significantly different from the 3-month level. **Trans individuals treated with testosterone:** Hemoglobin and hematocrit increased to stable levels by 6 months following T All later time points were not significantly different from the 6-month level
Boekhout-Berends et al., 2023 (48)	Netherlands	Retrospective, cohort No control group	Baseline, after 1-year GAHT and post-gonadectomy (3.5 years after GAHT)	E2 ($n = 1178$) T ($n = 1023$) Sex steroid concentrations confirmed within target ranges	**Trans individuals treated with estradiol:** Hemoglobin reference ranges, g/dL: Baseline vs 12 months vs post-gonadectomy: 13.1–17.2 vs 12.3–15.8 vs 11.6–15.5 Hematocrit, %:

Continued

Table 2. (continued)

Reference	Country	Study type	Duration	Treatment regimen (number); sex steroid concentrations	Results
					Baseline vs 12 months vs post-gonadectomy: 39–50 vs 35–46 vs 34–46
					Trans individuals treated with testosterone:
					Hemoglobin, g/dL:
					Baseline vs 12 months vs post-gonadectomy: 11.1–15.5 vs 11.9–17.4 vs 11.4–17.6
					Hematocrit, %:
					Baseline vs 12 months vs post-gonadectomy: 34–46 vs 38–52 vs 36–52

Abbreviations: CM, cisgender man; CW, cisgender woman; E2, estradiol; IQR, interquartile range; T, testosterone.

[13 μmol/L (0–26)] in 370 trans people undergoing masculinizing GAHT but did not change [−0.05 mg/dL (95% CI, −0.16 to 0.05)] [−4 μmol/L (−14–4)] in 361 trans people undergoing feminizing GAHT.

A subsequent longitudinal study performed as part of the Amsterdam Cohort of Gender Dysphoria reported the results of 1178 trans people undergoing feminizing GAHT and 1023 trans people undergoing masculinizing GAHT. Individuals had laboratory values reported prior to initiation of GAHT, 1 year following GAHT, and following gonadectomy (3.5 years following GAHT). Trans people undergoing feminizing GAHT were treated with oral or transdermal estradiol with cyproterone acetate as antiandrogen, and trans people undergoing masculinizing GAHT were treated with transdermal or intramuscular testosterone. This study reported a decrease in creatinine levels in those undergoing feminizing GAHT and increase in creatinine in those undergoing masculinizing GAHT at both 1 and 3.5 years (48).

Serum creatinine is affected by changes in lean body mass, which also changes during feminizing and masculinizing GAHT. Thus, changes in creatinine-derived eGFR may not reflect true changes in GFR and alternative biomarkers not affected by lean body mass may more accurately assess renal function in trans people undergoing GAHT. A recent study has evaluated changes in cystatin C following initiation of GAHT and demonstrated that cystatin C-based eGFR increased with feminizing GAHT and decreased with masculinizing GAHT (60).

Studies evaluating the influence of GAHT on renal function are shown in Table 3.

CLINICAL IMPLICATIONS

Significant changes in lean body mass are seen from 3 months following initiation of GAHT (62), which is associated with changes in serum creatinine concentration and eGFR in some but not all studies. For those receiving feminizing GAHT, which typically induces a decrease in muscle mass and gain in fat mass, the female CKD-EPI formula would be most appropriate. Conversely, for those receiving masculinizing GAHT, which typically induces an increase in muscle mass and decrease in fat mass, the male CKD-EPI formula would be most appropriate after 3 months GAHT.

In clinical situations in which accurate assessment of renal function is necessary such as consideration for transplant or renal replacement therapy, it may be more appropriate to use measures of renal function that are less affected by sex and not affected by muscle mass, including 24-hour urinary creatinine clearance, urinary inulin clearance, or serum cystatin c concentration. Notably, only serum cystatin c concentration has been evaluated in trans populations (60).

Table 3. Summary of studies investigating renal function in transgender individuals.

Reference	Country	Design	Duration	Number	Results
van Kesteren et al., 1998 (58)	Netherlands	Prospective, cohort No control group	45.5 (9.7) months	E2 ($n = 20$) T ($n = 19$) Sex steroid concentrations confirmed within target ranges	**Trans individuals treated with estradiol:** Creatinine, mg/dL: Baseline vs 1 year vs final measurement mean (SD): 0.92 (0.08) vs 0.87 (0.08) vs 0.88 (0.08), $P = 0.048$ **Trans individuals treated with testosterone:** Creatinine, mg/dL: Baseline vs 1 year vs final measurement [mean (SD), mg/dL]: 0.81 (0.09) vs 0.89 (0.10) vs 0.96 (0.07), $P < 0.001$
Wierckx et al., 2014 (39)	Belgium, Norway	Prospective, cohort No control group	12 months	E2 ($n = 53$) T ($n = 53$) Sex steroid concentrations confirmed within target ranges	**Trans individuals treated with estradiol:** Creatinine, mg/dL: Baseline vs 12 months (mean (SD), mg/dL): Oral: 0.9 (0.1) vs 0.8 (0.1), $P = 0.001$ Transdermal: 0.93 (0.1) vs 0.85 (0.1), $P = 0.011$ **Trans individuals treated with testosterone:** Creatinine, mg/dL: Baseline vs 12 months [mean (SD), mg/dL]: 0.74 (0.1) vs 0.84 (0.1), $P < 0.001$
Roberts et al., 2014 (41)	United States	Cross-sectional CW and CM controls	N/A >6 months E2 (median 4 years)	E2 ($n = 55$) CW ($n = 20$) CM ($n = 20$) Sex steroid concentrations not reported	**Trans individuals treated with estradiol:** Creatinine, mg/dL: Trans people vs CM vs CW (2.5th–97.5th percentile, mg/dL): 0.55–1.3 vs 0.73–1.3 vs 0.65–1.0
Fernandez et al., 2016 (36)	United States	Retrospective, cohort No control group	6–18 months	E2 ($n = 33$) T ($n = 19$) Sex steroid	**Trans individuals treated with estradiol:** Creatinine, mg/dL: Baseline vs 3–6 months vs 6–18 months [mean

Continued

Table 3. (continued)

Reference	Country	Design	Duration	Number	Results
				concentrations not reported	(SE), mg/dL]: 0.90 (0.03) vs 0.85 (0.03) vs 0.83 (0.03), $P < 0.05$ (baseline vs 3–6 months) **Trans individuals treated with testosterone:** Creatinine, mg/dL: Baseline vs 3–6 months vs 6–18 months [mean (SE), mg/dL]: 0.73 (0.03) vs 0.87 (0.04) vs 0.82 (0.04), $P < 0.01$
Vita et al., 2018 (33)	Italy	Retrospective, cohort No control group	3–6 months	E2 ($n = 21$) T ($n = 11$) Sex steroid concentrations confirmed within target ranges	**Trans individuals treated with estradiol:** Creatinine, mg/dL: Baseline vs 3–6 months [mean (SD)]: 0.79 (0.10) vs 0.79 (0.10), $P = NS$ **Trans individuals treated with testosterone:** Creatinine, mg/dL: Baseline vs 3–6 months [mean (SD)]: 0.70 (0.10) vs 1.00 (0.10), $P < 0.01$
SoRelle et al., 2019 (44)	United States	Retrospective, cohort No cisgender control group	N/A Groups: No GAHT vs >6 months GAHT	E2 ($n = 133$) vs no E2 ($n = 62$) T ($n = 89$) vs no T ($n = 87$) Sex steroid concentrations reported	**Trans individuals treated with estradiol:** Creatinine, mg/dL: Baseline vs post-treatment groups [median (IQR)]: 0.98 (0.68–1.3) ($n = 86$) vs 0.86 (0.64–1.25) ($n = 131$), $P < 0.0001$ (Cohen's $d = 0.59$) **Trans individuals treated with testosterone:** Creatinine, mg/dL: Baseline vs post-treatment groups [median (IQR)]: 0.74 (0.56–0.99) ($n = 57$) vs 0.90 (0.62–1.41) ($n = 79$), $P < 0.0001$ (Cohen's d 1.32)
Humble et al., 2019 (34)	United States	Retrospective chart review No control group	12 months	E2 ($n = 152$) T ($n = 150$) Sex steroid	**Trans individuals treated with estradiol:** Creatinine, mg/dL: Baseline vs 6 months ($n = 66$) [mean (SD)]: 0.92

Continued

Table 3. (continued)

Reference	Country	Design	Duration	Number	Results
				concentrations confirmed within target ranges	(0.14) vs 0.91 (0.16), $P = 0.56$ Baseline vs 12 months ($n = 49$): 0.93 (0.15) vs 0.89 (0.15), $P < 0.05$ **Trans individuals treated with testosterone:** Creatinine, mg/dL: Baseline vs 6 months ($n = 25$) [mean (SD)]: 0.77 (0.10) vs 0.90 (0.12), $P < 0.001$ Baseline vs 12 months ($n = 15$): 0.77 (0.12) vs 0.89 (0.13), $P < 0.001$
Scharff et al., 2019 (61)	Netherlands, Belgium, Norway, Italy	Prospective, cohort No control group	12 months	E2 ($n = 249$) T ($n = 278$) Sex steroid concentrations confirmed within target ranges	**Trans individuals treated with estradiol** Creatinine, mg/dL: Baseline vs during hormone therapy: 0.89 (0.12) vs 0.83 (0.12) **Trans individuals treated with testosterone** Creatinine, mg/dL: Baseline vs during hormone therapy [mean (SD)]: 0.75 (0.10) vs 0.88 (0.12)
Allen et al., 2021 (47)	United States	Retrospective, cohort No control group	60 months	E2 ($n = 126$) T ($n = 91$) Sex steroid concentrations not reported	**Trans individuals treated with estradiol:** Variable decreases in creatinine compared with baseline at 12, 42, 54, and 60 months. None of the follow-up time intervals were significantly different from 3 months. **Trans individuals treated with testosterone:** Significant increase in creatinine into the cisgender male reference range at 9 months. Creatinine was then stable over 5 years.
Maheshwari et al., 2022 (56)	United States	Retrospective, cohort No control group	12 months	E2 ($n = 84$) T ($n = 24$) Sex steroid	**Trans individuals treated with estradiol:** Creatinine, mg/dL: Baseline vs 3 months vs 6 months vs 12 months [median (IQR)]: 0.93 (0.86–1.07) vs 0.9 (0.8–

Continued

Table 3. (continued)

Reference	Country	Design	Duration	Number	Results
				concentrations reported	1.0) vs 0.83 (0.79–0.99) vs 0.86 (0.79–1.00), $P < 0.01$ at 12 months **Trans individuals treated with testosterone:** Creatinine, mg/dL: Baseline vs 3 months vs 6 months vs 12 months [median (IQR)]: 0.81 (0.8–0.9) vs 0.85 (0.74–0.95) vs 0.9 (0.85–1.11) vs 0.93 (0.84–1.08), $P < 0.01$ at 12 months
Humble et al., 2022 (57)	United States	Cross-sectional No control group	N/A	E2 ($n = 93$) T ($n = 82$) Sex steroid concentrations not reported	**Trans individuals treated with estradiol:** creatinine unchanged from reference range for cisgender men **Trans individuals treated with testosterone:** creatinine essentially the same as cisgender male reference range, higher than cisgender female reference range
Boekhout-Berends et al., 2023 (48)	Netherlands	Retrospective, cohort No control group	Baseline, after 1-year GAHT and post-gonadectomy (3.5 years after GAHT)	E2 ($n = 1178$) T ($n = 1023$) Sex steroid concentrations confirmed within target ranges	**Trans individuals treated with estradiol:** Creatinine reference ranges, mg/dL: Baseline vs 12 months vs post-gonadectomy: 0.66–1.14 vs 0.62–1.07 vs 0.62–1.13 **Trans individuals treated with testosterone:** Creatinine reference ranges, mg/dL: Baseline vs 12 months vs post-gonadectomy: 0.58–0.98 vs 0.66–1.13 vs 0.65–1.20
Van Eeghen et al., 2023 (60)	ENIGI	Prospective, cohort No control group	12 months	E2 ($n = 260$) T ($n = 285$) Sex steroid	**Trans individuals treated with estradiol:** cystatin C decreased by mean 0.069 mg/L (95% CI, 0.049 to 0.089), corresponding with

Continued

Table 3. (continued)

Reference	Country	Design	Duration	Number	Results
				concentrations confirmed within target ranges	a 7 mL/min/1.73 m^2 increase in eGFR. **Trans individuals treated with testosterone:** cystatin C increased by mean 0.052 mg/L (95% CI, 0.031 to 0.072), corresponding with a 6 mL/min/1.73 m^2 decrease in eGFR.

Abbreviations: CI, confidence interval; CM, cisgender man; CW, cisgender woman; E2, estradiol; ENIGI, European Network for the Investigation of Gender Incongruence; IQR, interquartile range; T, testosterone.

From a practical perspective, we recommend that the treating clinician specify the desired sex-specific reference range on the laboratory request. This will allow reporting of the clinically appropriate reference range and permit evaluation of longitudinal changes in renal function.

Cardiac Biomarkers

CARDIAC TROPONIN

Cardiac troponins (cardiac troponin T and cardiac troponin I cTnI) are cardiac regulatory proteins that control the calcium-mediated interaction between actin and myosin. Cardiac troponin is released from damaged cardiomyocytes and is one of the most common biomarkers used in the prediction of acute coronary syndrome. Sex-specific 99th percentile reference intervals are subtly higher in people recorded male at birth, presumably due to larger cardiac mass and subclinical coronary artery disease (63). Large population-based studies have also shown that left ventricular mass correlates with body weight, lean body mass, and fat mass (64). Despite this, there remains uncertainty regarding the utility of sex-specific reference ranges for high-sensitivity cardiac troponin (hs-cTn) on management or clinical outcomes (65). Importantly, the use of sex-specific cardiac troponin reference ranges was not found to affect clinical outcomes in a population of trans people with suspected acute coronary syndrome (66).

Within the trans population, a cross-sectional study evaluated cardiac troponin concentrations in 79 trans people prescribed testosterone and 93 trans people prescribed estradiol from the Unites States. Median (IQR) Abbott high-sensitivity cardiac troponin I observed in trans people prescribed testosterone was 0.9 (0.6–1.7) ng/L and 0.6 (0.3–1.0) ng/L in trans people prescribed estradiol (67). However, in another cross-sectional study that enrolled 48 trans people undergoing feminizing hormone therapy [median duration 6 years (3.3–17)] and 15 undergoing masculinizing hormone therapy [median duration 3.6 years (2.1–5.2)] (68), there was no difference in cardiac troponin T (Roche) or I (Beckman or Architect) between those on masculinizing and feminizing GAHT (Table 4).

B-TYPE NATRIURETIC PEPTIDE

B-type natriuretic peptides (BNP) have roles in cardiovascular remodeling and volume homeostasis and are also released from damaged cardiomyocytes. BNP and N-terminal pro-BNP levels are higher in women than in men in the general population (70). Two cross-sectional studies have evaluated BNP concentrations in trans populations. Consistent with the cisgender population, trans people prescribed estradiol had higher BNP

Table 4. Summary of studies investigating cardiac biomarkers in transgender individuals.

Reference	Country	Design	Duration	Number	Results
Deischinger et al., 2023 (69)	Austria	Cross-sectional CM and CW control groups	N/A Median 1.4 years (1.0–2.2) E2, median 1.1 years (1.0–2.0) T	E2 ($n = 22$) CM ($n = 16$) T ($n = 16$) CW ($n = 17$)	**Pro-BNP:** Median (IQR) pro-BNP (pg/mL) in trans people prescribed E2 was higher than CM [36.6 (33.0–60.9) vs 11.75 (2.5–31.0)], $P < 0.01$ Median (IQR) pro-BNP in trans people prescribed T was lower than CW [9.3 (2.5–39.35) vs 42.2 (28–59.9)], $P < 0.05$
Boone et al., 2023 (68)	United States	Cross-sectional No control group	N/A Median 6 years E2, median 3.6 years T	E2 ($n = 48$) T ($n = 15$)	**hs-cTn:** Roche TnT (ng/L): E2 4.3 (2.9–6.6) vs T 3.1 (3–4.7), $P = 0.07$ Beckman TnI: E2 2(2–3.7) vs T 2 (2–2.4), $P = 0.24$ Architect TnI STAT: E2 1.7 (1.7–2.2) vs T 1.7 (1.7–1.7), $P = 0.29$
Greene et al., 2022 (67)	United States	Cross-sectional No control group	N/A At least 12 months GAHT	E2 ($n = 93$) T ($n = 79$)	**hs-cTn:** Median (IQR) hs-cTn (ng/L) in trans people prescribed E2 was lower than that in people prescribed T [(0.6 (0.3–1.0) vs 0.9 (0.6–1.7), $P < 0.001$] **NT-proBNP:** Median (IQR) NT-proBNP (ng/L) in trans people prescribed E2 was higher than that in people prescribed T [49 (32–86) vs 17 (13–27), $P = 0.001$]

Abbreviations: CM, cisgender man; CW, cisgender woman; E2, estradiol; hs-cTn, high-sensitivity cardiac troponin; IQR, interquartile range; NT-proBNP, N-terminal pro-brain natriuretic peptide; T, testosterone. Creatinine mg/dL = μmol/L × 88.42.

concentrations compared to cisgender men (69) and transgender men (67), and individuals prescribed testosterone had lower BNP than cisgender women (69) and transgender women (67) (Table 4).

There are currently no studies evaluating longitudinal changes in cardiac biomarkers following initiation of GAHT. Studies evaluating the influence of GAHT on cardiac biomarkers are shown in Table 4.

CLINICAL IMPLICATIONS

There is currently insufficient data to draw an inference regarding the appropriate reference ranges for cardiac biomarkers in people using GAHT. Emphasis must be placed on clinical history and examination, electrocardiogram changes, and serial trajectory of cardiac biomarkers if levels fall between sex-specific reference ranges.

Prostate-Specific Antigen

There are limited data examining the effect of feminizing GAHT on prostate-specific antigen (PSA). However, a recent retrospective cohort study reported PSA results in 210 patients who underwent 852 PSA tests (71). Mean age was 60 years and median duration of estradiol treatment was 5 years. Median PSA of the cohort was 0.02 ng/mL (0–0.2) and the 95th percentile

Table 5. Recommended reference ranges for trans individuals undergoing ≥3 months GAHT.

	Recommended reference range	
Laboratory test	Affirmed gender	Sex recorded at birth
Estradiol	✓	
Total testosterone	✓	
Hemoglobin/ hematocrit	✓	
Creatinine	✓	
Cardiac troponin	Insufficient data	Insufficient data
BNP	Insufficient data	Insufficient data
PSA[a]		✓ (more aligned with men undergoing ADT)

[a]Only relevant for people with a prostate; ADT, androgen deprivation therapy.

value was 0.6 ng/mL (71). Notably, this is lower than that in cisgender men without prostate cancer (72) and more closely aligns with hypogonadal cisgender men undergoing androgen deprivation therapy for prostate cancer (73). In keeping with this, a nationwide cohort study has reported a lower incidence of prostate cancer in trans people undergoing feminizing GAHT ($n = 2281$) compared to expected cases based on age specific incidence numbers in the general male population (standardized incidence ratio 0.20, 95% CI 0.08–0.42) (74). Six individuals in this cohort had a diagnosis of prostate adenocarcinoma with a median PSA of 18 ng/mL (range, 5–1722).

CLINICAL IMPLICATIONS

There are insufficient data evaluating reference ranges to permit recommendation of a specific PSA cutoff in trans people established on feminizing GAHT. As such, clinicians should make individualized decisions based on clinical history and physical examination to inform the need for PSA monitoring and/or follow-up imaging.

Recommendations

Given that serum sex steroid concentrations and resultant changes to body composition begin to occur within 3 months of initiation of GAHT, we recommend that the reference range of the individual's affirmed gender should be used for tests with sex-specific reference ranges after 3 months GAHT (Table 5). Individualized interpretation will still need to occur, particularly for individuals with recent GAHT commencement or for those treated with low-dose GAHT in whom sex-specific parameters could be between the male and female reference ranges. Notably, some trans individuals do not desire GAHT, and the reference range of their sex recorded at birth should be used.

Treating clinicians should specify whether the male or female reference range should be reported on the laboratory request. If no gender marker is available, we suggest that clinicians are contacted to ensure the correct reference ranges are reported. We acknowledge that this might not be feasible in high-volume settings, so clinicians must take note of the reference range reported and notify the laboratory if an alternative reference range is required. Ultimately, there is no "one size fits all" and interpretation should be individualized for each patient based on the relevant clinical information. Dual reporting of both male and female reference ranges is another potential option; however, laboratory information system barriers exist, and this has the potential to lead to confusion (75).

Ideally, system fields should include both legal and preferred name, gender identity, sex recorded at birth, and pronouns. Signage should indicate the need to obtain sensitive information including sex recorded at birth (which may be distressing or induce dysphoria for some but is of clinical relevance), which will assist the laboratory staff in providing an affirming space for trans people.

Future Directions

Limitations of the current evidence-base include that most studies are cross-sectional with small sample sizes in different cohorts of trans individuals. Similarly, the methods used to report findings differed between studies. Larger, prospective studies following trans people following initiation of different GAHT regimens are required, as well as an evaluation of biological variation. Additionally, current data are based on binary trans people treated with full-dose GAHT, and further research should evaluate changes in laboratory parameters in binary and nonbinary individuals seeking low-dose GAHT. Finally, further research is required on other laboratory parameters with sex-specific reference ranges, including iron studies.

Conclusion

With increasing numbers of trans people seeking GAHT, clinicians, pathologists, and laboratory scientists may need to be able to interpret common laboratory results and appropriate reference ranges for trans individuals undergoing GAHT. We propose that once

individuals have commenced GAHT, in general, the reference range of the affirmed gender should be reported for laboratory tests with sex-specific reference intervals. Treating clinicians should provide this information by using the corresponding gender marker on laboratory requests or provide relevant history to ensure the appropriate laboratory reference ranges are reported and patient care is optimized.

Nonstandard Abbreviations: GAHT, gender-affirming hormone therapy; GnRH, gonadotropin releasing hormone; eGFR, estimated glomerular filtration rate; BNP, B-type natriuretic peptide; PSA, prostate-specific antigen.

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