Clinical Research Article

Longitudinal Changes in Hematologic Parameters Among Transgender People Receiving Hormone Therapy

Ana Antun,[1,2] Qi Zhang,[3] Shalender Bhasin,[4,5] Andrew Bradlyn,[6] W. Dana Flanders,[3] Darios Getahun,[7] Timothy L. Lash,[3] Rebecca Nash,[3] Douglas Roblin,[8] Michael J. Silverberg,[9] Vin Tangpricha,[1,2] Suma Vupputuri,[8] and Michael Goodman[3]

[1]Emory University, School of Medicine, Atlanta, Georgia 30322; [2]The Atlanta VA Medical Center, Atlanta, Georgia 30033; [3]Rollins School of Public Health, Emory University, Atlanta, Georgia 30322; [4]Brigham and Women's Hospital, Boston, Massachusetts 02115; [5]Harvard School of Medicine, Boston, Massachusetts 02115; [6]Center for Research and Evaluation, Kaiser Permanente Georgia, Atlanta, Georgia 30309; [7]Department of Research and Evaluation, Kaiser Permanente Southern California, Pasadena, California 91101; [8]Mid-Atlantic Permanente Research Institute, Kaiser Permanente Mid-Atlanta States, Rockville, Maryland 20852; and [9]Division of Research, Kaiser Permanente, Northern California, Oakland, California 94612

ORCiD numbers: 0000-0002-3982-6200 (S. Bhasin); 0000-0002-5240-5195 (T. L. Lash); 0000-0001-9322-1395 (M. J. Silverberg); 0000-0001-6956-6879 (M. Goodman).

Abstract

Context: The effect of gender-affirming hormone therapy (HT) on erythropoiesis is an area of priority in transgender health research.

Objective: To compare changes in hematologic parameters and rates of erythrocytosis and anemia among transgender people to those of cisgender controls.

Design: Longitudinal observational study.

Participants and Setting: We compared 559 transfeminine (TF) and 424 transmasculine (TM) people enrolled in 3 integrated health care systems to matched cisgender referents.

Interventions and Outcome: Hormone therapy receipt was ascertained from filled prescriptions. Hemoglobin (Hb) and hematocrit (Hct) levels were examined from the first blood test to HT initiation, and from the start of HT to the most recent blood test. Rates of erythrocytosis and anemia in transgender participants and referents were compared by calculating adjusted hazard ratios and 95% confidence intervals (CI).

Results: In the TF group, there was a downward trend for both Hb and Hct. The corresponding changes in the TM cohort were in the opposite direction. TM study participants experienced a 7-fold higher rate (95% CI: 4.1–13.4) of erythrocytosis relative to matched

cisgender males, and an 83-fold higher rate (95% CI: 36.1–191.2) compared to cisgender females. The corresponding rates for anemia were elevated in TF subjects but primarily relative to cisgender males (hazard ratio 5.9; 95% CI: 4.6–7.5).

Conclusions: Our results support previous recommendations that hematological parameters of transgender people receiving HT should be interpreted based on their affirmed gender, rather than their sex documented at birth. The clinical significance of erythrocytosis following testosterone therapy, as well as anemia following feminizing HT, requires further investigation.

Key Words: transgender, hormone therapy, hemoglobin, hematocrit, longitudinal

Masculinizing and feminizing hormones constitute an important component of medical gender affirmation among transgender people [1, 2]. For transfeminine (TF) individuals, the gender-affirming hormone therapy (HT) usually includes estrogens, often in combination with antiandrogens [3]. For transmasculine (TM) individuals, hormonal gender affirmation involves the administration of testosterone [4]. Short- and long-term benefits and risks of HT represent an emerging field of clinical research, with many open questions [5–9].

A specific area of both research and practical interest is the effect of gender-affirming HT on various laboratory parameters that can occur following the administration of feminizing and masculinizing hormones. In particular, HT use is expected to stimulate erythrogenesis, as evidenced by elevated Hb or Hct levels in TM patients, and to produce an opposite effect in TF persons undergoing gender affirmation treatment [10–12].

Although changes in erythrogenesis after initiation of gender-affirming therapy are expected to be mostly physiological, the laboratory values during the transition period may be interpreted as abnormal if based on inappropriate reference ranges [12, 13]. In addition, there is a concern that testosterone therapy may produce clinically significant erythrocytosis in TM patients [9, 14].

Previous clinical studies consistently reported significant increases in Hct and Hb following the initiation of testosterone therapy among TM patients, whereas feminizing hormone therapy was shown to have an opposite effect [15–21]. On the other hand, relatively limited data are available on the temporal trajectories of Hb and Hct changes among TM and TF subjects receiving HT relative to cisgender referent groups. Moreover, little information is available regarding the incidence of erythrocytosis and anemia in relation to gender-affirming hormone therapy, especially over extended periods of time.

To help with closing these knowledge gaps, we used data from a large cohort study nested in integrated health care systems that allowed access to electronic health records (EHR) and permitted efficient identification and follow-up of hard-to-reach population subgroups, such as transgender people. The aims of the present study were to examine longitudinal changes in the main hematologic parameters among TF and TM persons before and after HT initiation, and to compare trajectories of these parameters, as well rates of erythrocytosis and anemia, to those of matched cisgender individuals.

Methods

Cohort ascertainment and data collection

The Study of Transition, Outcomes and Gender (STRONG) aimed to examine the health status of transgender people and to compare various measures of morbidity among transgender participants to those of matched cisgender referents. STRONG cohort members were identified between 2006 and 2014 from the EHR of individuals enrolled in Kaiser Permanente health plans in Northern California, Southern California, and Georgia.

The details of STRONG cohort ascertainment and data collection were described in previous publications [22, 23]. Briefly, each transgender cohort member was identified in the EHR based on diagnostic codes and free-text keywords, and up to 20 cisgender referents (10 male and 10 female) were matched to each transgender cohort member based on birth year (within 5-year groups), race/ethnicity, study site, and enrollment year of the first recorded evidence of transgender status (index date). A unique cluster identification number was used to link the transgender participants to their matched cisgender reference group. Additional EHR data linkages for each study participant were used to ascertain new diagnoses, procedures, laboratory test results, and pharmacy prescriptions. The Institutional Review Boards at Kaiser Permanente sites and at Emory University (coordinating center) reviewed and approved all study elements with exemption of informed consent.

The current study focused on transgender participants in the hormone initiation group, which included individuals who started HT at Kaiser Permanente after the index date. Each transgender cohort member was categorized as TF or TM, based on sex documented at birth. The TM and TF assignment methodology was described and validated previously [23, 24]. The receipt of gender-affirming HT was ascertained based on filled prescription information documented in the pharmacy records.

The transgender cohort members and their matched referents were eligible for analysis if they were at least 18 years old at the index date and underwent at least 1 blood test both before and after the date of HT initiation. As not all cisgender participants met these criteria, the average number of referents matched to a transgender cohort member was lower than 20. The dependent variables of interest included 2 hematologic parameters (Hct and Hb) known to be influenced by estrogen and testosterone, and 1 parameter (platelet count) that is not expected to be affected by sex hormones. The platelet count in this case served to test a prespecified "falsification hypothesis," which is defined as a claim that is believed to be highly unlikely and, if found to be incorrect, can lend additional credibility to the main association under investigation [25]. Each hematologic parameter of interest was ascertained from laboratory records that included the measured values and the dates of the blood tests.

The time under observation was divided into 2 intervals: from the first blood test to immediately before HT initiation and from the start of HT to the most recent blood test. The date of the first filled HT prescription was assigned a value of 0; thus pre-HT time had negative values and time post-HT initiation had positive values (in years).

Statistical analysis

All data analyses were conducted using SAS, version 9.4 (SAS Institute, Cary, North Carolina). The analyses included 3 components: an overall comparison of averages before and after HT, a longitudinal analysis of values in relation to HT receipt, and a time-to-event evaluation of erythrocytosis rates. Each analysis included 2 comparisons for TM and TF, performed separately relative to the respective cisgender male and cisgender female referent groups.

For the first analysis, values of laboratory measures for each participant were averaged before and after HT initiation, and means and standard deviations were used to describe the distributions of the average results. We then examined the distribution of laboratory values for each hematologic parameter of interest in TM and TF participants before and after HT initiation, and compared these distributions to those of matched cisgender reference participants. The results of these comparisons were presented as histograms and reported as differences of means and the corresponding 95% confidence intervals (CIs).

We also calculated the 2.5^{th} and 97.5^{th} percentiles as the potential reference limits of the parameters of interest. For consistency with previous research [26], the reference limits were calculated after at least 1 year following HT initiation, and 95% CIs for each reference limit were estimated using bootstrapping procedure with 1000 iterations.

For the longitudinal analyses, we used linear mixed models to characterize the changes in laboratory measures in relation to HT use. The linear mixed models accounted for within-subject correlation in repeated laboratory measures and permitted modeling of heterogeneity in the changes of hematological parameters over time across individuals by adding random time slopes. We applied linear splines for the time variable, with a knot at HT initiation. The scalar form of the linear mixed models is as follows:

$$\text{Hct}_{ij} = \beta_0 + b_{0i} + \beta_1\,\text{trans}_{ij} + \beta_2\,\text{time\_ht}_{ij} + b_{1i}\text{time\_ht}_{ij}$$
$$+ \beta_3\text{posttime}_{ij} + b_{2i}\text{posttime}_{ij} +$$
$$\beta_4\text{trans}_{ij} * \text{time\_ht}_{ij} + \beta_5\text{trans}_{ij} * \text{posttime}_{ij} + e_{ij}$$

where Hct_{ij} is the Hct value from measurement j, of subject I; $trans_{ij}$ is the variable indicating the transgender (vs cisgender) status; $time\_ht_{ij}$ is the re-centered time variable (negative prior to HT and positive after HT); $posttime_{ij}$ is the time interval from HT initiation to the date of the laboratory measure; $trans_{ij} * time\_ht_{ij}$ and $trans_{ij} * posttime_{ij}$ are interaction terms reflecting the difference in slopes between transgender and cisgender subjects before and after HT initiation, respectively; b_{0i} is the random intercept for subject i; b_{1i} is the random pre-HT slope over time for subject i; and the sum $b_{1i} + b_{2i}$ is the random post-HT slope for subject i.

The results of the models were expressed as linear regression coefficients and the corresponding 95% CIs. The slopes of changes in laboratory values were plotted for easier visualization of results.

We assessed the potential clinical significance of the longitudinal changes in the third (time-to-event) analysis, which evaluated the incidence of erythrocytosis and anemia following HT initiation. Erythrocytosis is typically defined as Hct above 52% for men and above 48% for women, and anemia is defined as Hct below 42% for men and below 36% for women [17, 27, 28]. Thus, in the time-to-event analyses that compared transgender

participants on HT to cisgender men, we applied the Hct cutoffs of >52% and <42% to define erythrocytosis and anemia, respectively. In the corresponding analyses that used cisgender females as the reference category, the cutoff for erythrocytosis was >48% and the cutoff for anemia was <36%. In order to focus on incident events, each time-to-event analysis for erythrocytosis included only those participants whose measured Hct levels never exceeded the predetermined cutoff before HT initiation, which served as the start of follow-up. Similarly, analysis for incident anemia were limited to individuals whose Hct levels before HT initiation were never below the cutoff. The follow-up extended from HT initiation until the first occurrence of erythrocytosis, disenrollment from the Kaiser Permanente health plan for more than 90 days, death, or the end of the study period (November 30, 2016). Matched referents were assigned the same date of start of follow-up.

The rates of erythrocytosis and anemia occurrence in transgender cohort members and matched referents were compared using Cox proportional hazards models. As incident erythrocytosis and incident anemia can only be diagnosed in participants who had at least 1 blood test during follow-up, there is an opportunity for selection bias if transgender persons on HT are more likely to be monitored than controls. To account for this possibility, we used weighted Cox models where the weights for the models represented inverse probabilities of having a blood test during follow-up. The probabilities of having a blood test were obtained from a separate logistic model, which included all cohort members who were eligible for inclusion in the analysis. The binary dependent variable in the logistic model was defined as having at least 1 blood test during follow-up and independent variables included age, transgender or referent status, race/ethnicity, study site, and the most recent Hct value prior to HT initiation.

Each weighted Cox model had 2 versions. Model 1 was stratified on cluster ID to account for matching on age, race/ethnicity, and study site. Model 2 was the same as Model 1, but also adjusted for the most recent Hct result before HT initiation. The results of all Cox models were expressed as hazard ratios (HR) and the corresponding 95% CIs. Proportional hazard assumptions were tested by examining log minus log survival plots for each variable in the model and by performing the goodness of fit test using Schoenfeld residuals [29].

Results

A total of 559 TF and 424 TM cohort members met the inclusion criteria. Compared to TF study participants, TM were younger and included slightly higher proportions of non-Hispanic Whites (Table 1).

As shown in Fig. 1, the Hct distribution among TF study participants before estrogen receipt was very similar to the distribution of cisgender men; however, after estrogen initiation, the distribution became similar to that of cisgender women. The shift among 424 TM was less pronounced because the Hct distribution in TM even before HT initiation was between the distributions observed in cisgender men and women. Once on HT, the Hct distributions in TM and cisgender men appeared almost the same (Fig. 2). Similar shifts were observed for Hb (data not shown). By contrast, no discernable differences or HT-related changes were observed for platelet counts (data not shown).

The longitudinal analyses of Hct levels in the TF cohort demonstrated a downward trend, with inflection at the time of HT initiation (Fig. 3). The model-derived average Hct of TF participants was approximately 43% to 45% 20 years before HT receipt and reached 37% 10 years after HT initiation. The corresponding longitudinal changes in the TM cohort demonstrated continuously increasing Hct levels even before HT initiation, without an identifiable inflection point (Fig. 4). The predicted average Hct level started around 35% to 38% 20 years before HT initiation, but reached 48% by 10 years of HT use. The patterns of change in Hb concentrations were similar to those observed for Hct among TF and TM study participants (data not shown).

Following at least a year of feminizing HT, the 2.5th percentile of Hct among TF was 34.1 (95% CI: 31.5, 36.7) and the 97.5th percentile was 47.6 (95% CI: 46.8, 48.4). Similarly, the 2.5th and 97.5th percentiles of Hct among TM after at least a year of HT were 37.4 (95% CI: 35.1, 39.7) and 52.3 (95% CI: 51.5, 53.1), respectively.

The time-to-event analysis results for erythrocytosis are presented in Table 2. The rate of erythrocytosis among TM study participants was 7.5 per 100 000 person-days (26 events) using the Hct cutoff of 52% and 39.3 per 100 000 person-days (91 events) using the cutoff of 48%. In all models, TM study participants experienced higher rates of erythrocytosis relative to their matched referents. The rates were especially elevated when compared to cisgender females with HR (95% CI) of 81.4 (41.1, 161.3) and 83.1 (36.1, 191.2) in Models 1 and 2, respectively. The corresponding estimates comparing TM to male controls were lower with HR of 5.5 (95% CI: 3.3, 9.2) for Model 1 and 7.4 (95% CI: 4.1, 13.4) for Model 2. Erythrocytosis was less common among TF cohort members, with only 3 and 15 cases observed, depending on the Hct cutoff, with corresponding rates of 0.4 and 2.4 per 100 000 person-days. Relative to reference cisgender

Table 1. Characteristics of the Transgender and Matched Reference Cohorts[a]

Participant Characteristics[b]	Transfeminine Cohort (n = 559)	Reference Males (n = 2388)[c]	Reference Females (n = 3240)	Transmasculine Cohort (n = 424)	Reference Males (n = 1309)	Reference Females (n = 2018)
Membership site						
KPNC	331 (59.2)	1500 (62.8)	2002 (61.8)	278 (65.6)	854 (65.2)	1319 (65.4)
KPSC	214 (38.3)	841 (35.2)	1185 (36.6)	142 (33.5)	445 (34.0)	682 (33.8)
KPGA	14 (2.5)	47 (2.0)	53 (1.6)	4 (0.9)	10 (0.8)	17 (0.8)
Race/ethnicity						
Non-Hispanic white	320 (57.3)	1475 (61.8)	1866 (57.6)	268 (63.2)	861 (65.8)	1279 (63.4)
Non-Hispanic black	48 (8.6)	186 (7.8)	304 (9.4)	51 (12.0)	151 (11.5)	270 (13.4)
Asian/Pacific Islander	62 (11.1)	246 (10.3)	344 (10.6)	33 (7.8)	101 (7.7)	146 (7.2)
Hispanic	104 (18.6)	414 (17.3)	625 (19.3)	64 (15.1)	175 (13.4)	290 (14.4)
Other/unknown	25 (4.5)	67 (2.8)	101 (3.1)	8 (1.9)	21 (1.6)	33 (1.6)
Age at index date, years						
18–25	155 (27.7)	414 (17.3)	674 (20.8)	164 (38.7)	374 (28.6)	632 (31.3)
26–35	141 (25.2)	463 (19.4)	804 (24.8)	150 (35.4)	429 (32.8)	770 (38.2)
36–45	112 (20.0)	519 (21.7)	694 (21.4)	60 (14.2)	239 (18.3)	308 (15.3)
46–55	77 (13.8)	465 (19.5)	509 (21.4)	40 (9.4)	211 (16.1)	237 (11.7)
>55	74 (13.2)	527 (22.1)	559 (17.3)	10 (2.4)	56 (4.3)	71 (3.5)
# Lab tests pre-HT, days						
1–2	297 (53.1)	1065 (44.6)	958 (29.6)	137 (32.3)	585 (44.7)	496 (24.6)
3–6	159 (28.4)	769 (32.2)	1094 (33.8)	144 (34.0)	436 (33.3)	600 (29.7)
>6	103 (18.4)	554 (23.2)	1188 (36.7)	143 (33.7)	288 (22.0)	922 (45.7)
# Lab tests on-HT, days						
1–2	164 (29.3)	1259 (52.7)	1422 (43.9)	156 (36.8)	910 (69.5)	1102 (54.6)
3–6	217 (38.8)	632 (26.5)	1046 (32.3)	181 (42.7)	282 (21.5)	625 (31.0)
>6	178 (31.8)	497 (20.8)	772 (23.8)	87 (20.5)	117 (8..9)	291 (14.4)
Hct average level pre-HT	42.8 (3.6)	43.6 (3.4)	38.6 (2.9)	42.3 (3.9)	43.8 (3.1)	38.2 (2.9)
Hct average level on-HT	40.7 (3.3)	43.6 (3.7)	39.0 (3.3)	44.8 (3.6)	44.0 (3.4)	38.7 (3.3)

Abbreviations: Hct, hematocrit; HT, hormone therapy; KPGA, Kaiser Permanente Georgia; KPNC, Kaiser Permanente Northern California; KPSC, Kaiser Permanente Southern California.

[a]Average number of matched referents to each transgender cohort member is <10 because data are limited to subjects with at least 1 blood test available before and after HT date.

[b]Calculated as n (%) for membership site, race/ethnicity, age, and number of lab tests, and as mean (standard deviation) for average Hct levels.

[c]Two missing values in the measure of Hct for reference men. Percentages may not add up to 100 due to rounding.

males, the HR for TF was 0.4 regardless of the model, but the CIs were wide. When compared to reference females, the rate of erythrocytosis was elevated in Model 1 (HR: 4.4; 95% CI: 2.4–8.61); however, the association was no longer observed after controlling for pre-HT Hct levels (Table 2).

As shown in Table 3, the rates of anemia among TM study participants were 28.6 per 100 000 person-days (22 events) using the Hct cutoff of 42% and 4.8 per 100 000 person-days (91 events) using the cutoff of 36%. Transmasculine study participants experienced higher rates of anemia relative to cisgender males (baseline Hct-adjusted HR: 3.1; 95% CI: 1.8–5.6) but lower rates compared with cisgender females (baseline Hct-adjusted HR: 0.3; 95% CI: 0.1–0.4). The corresponding rates for anemia were elevated in TF subjects, especially relative to cisgender males (HR: 5.9; 95% CI: 4.6–7.5), but less so

when compared to cisgender females (HR: 1.4; 95% CI: 1.1–1.8).

Discussion

Using data from the EHR-based STRONG cohort, we observed that both TM and TF participants experienced longitudinal changes in Hb and Hct in the hypothesized direction—upward in TM and downward in TF, both likely reflecting the effects of cross-sex hormones. In accordance with our prespecified "falsification hypothesis," no discernable changes were observed in platelet concentrations.

It is notable that towards the end of follow-up, the Hb and Hct values among TM and TF in our study were similar to those of cisgender male and female controls, respectively. This finding is in agreement with a recent cross-sectional
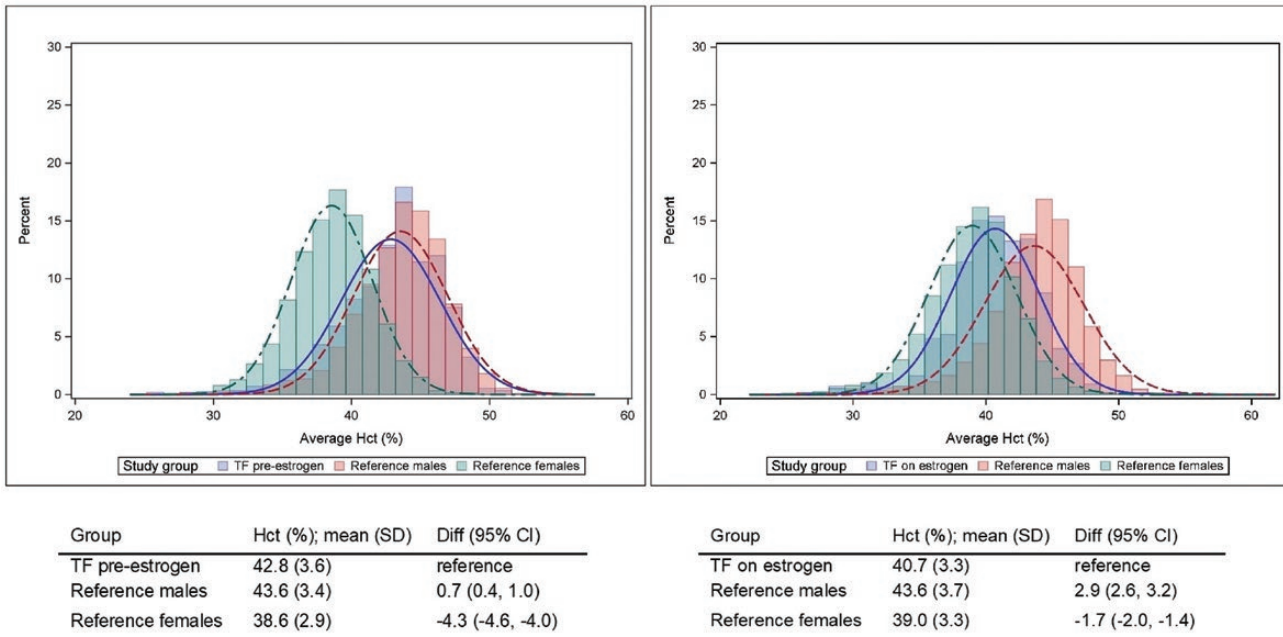
Group	Hct (%); mean (SD)	Diff (95% CI)
TF pre-estrogen	42.8 (3.6)	reference
Reference males	43.6 (3.4)	0.7 (0.4, 1.0)
Reference females	38.6 (2.9)	-4.3 (-4.6, -4.0)

Group	Hct (%); mean (SD)	Diff (95% CI)
TF on estrogen	40.7 (3.3)	reference
Reference males	43.6 (3.7)	2.9 (2.6, 3.2)
Reference females	39.0 (3.3)	-1.7 (-2.0, -1.4)

Figure 1. Average Hct values for TF individuals before and after HT initiation compared with matched referent males and females.

Group	Hct (%); mean (SD)	Diff (95% CI)
TM pre-testosterone	42.3 (3.9)	reference
Reference males	43.8 (3.1)	1.5 (1.1, 1.9)
Reference females	38.2 (2.9)	-4.1 (-4.5, -3.7)

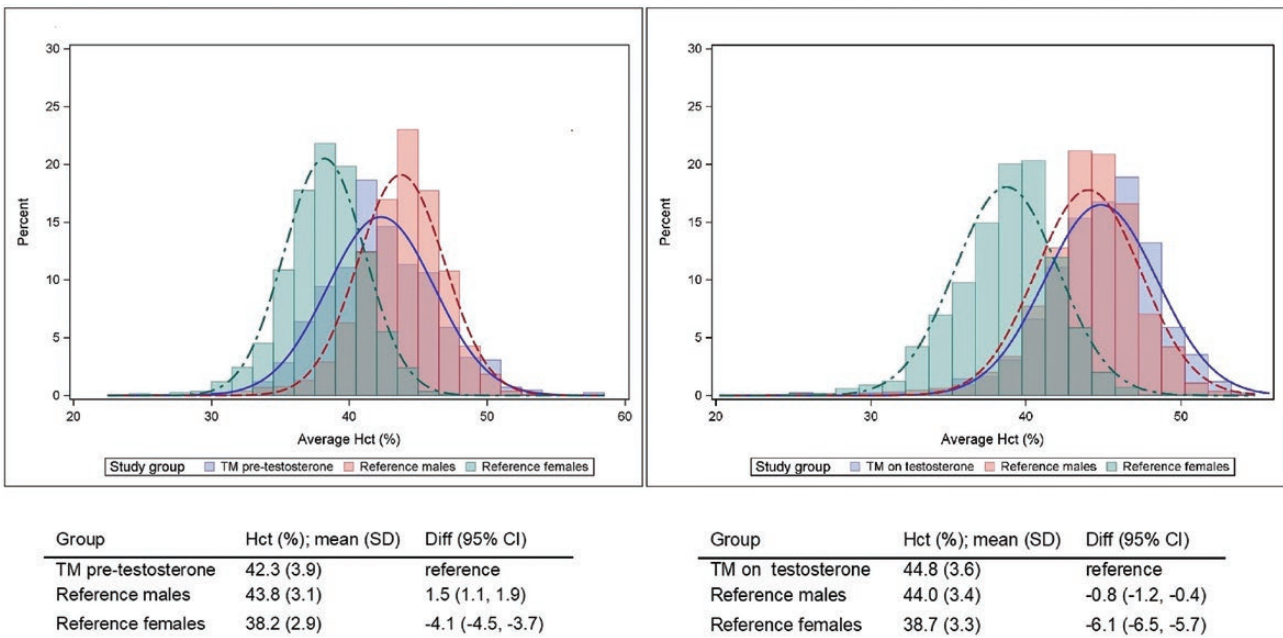| Group	Hct (%); mean (SD)	Diff (95% CI)
TM on testosterone	44.8 (3.6)	reference
Reference males	44.0 (3.4)	-0.8 (-1.2, -0.4)
Reference females	38.7 (3.3)	-6.1 (-6.5, -5.7)

Figure 2. Average Hct values for TM individuals before and after HT initiation compared to matched referent males and females.

analysis of the data from 2 primary care clinics that provide care to transgender patients and examined distributions of various hematologic parameters in 79 TM and 93 TF individuals [26]. At the time of data collection, all study participants were 18 years old or older and had received gender-affirming hormone therapy for at least 12 months. Most TM study participants were treated with intramuscular or subcutaneous testosterone. In the TF group, more than half received estrogen orally and about one-third

received estradiol valerate intramuscularly or subcutaneously. In addition to evaluating hematologic parameters, the study also measured serum hormone concentrations. In both groups, Hb was related to testosterone and estrogen concentrations, but the relationship was nonlinear and the Hb level appeared to reach a plateau above a certain threshold, which appeared to be around 2–4 ng/mL for serum testosterone and around 500 pg/mL for serum estradiol. The intervals between the 2.5th and 97.5th percentile
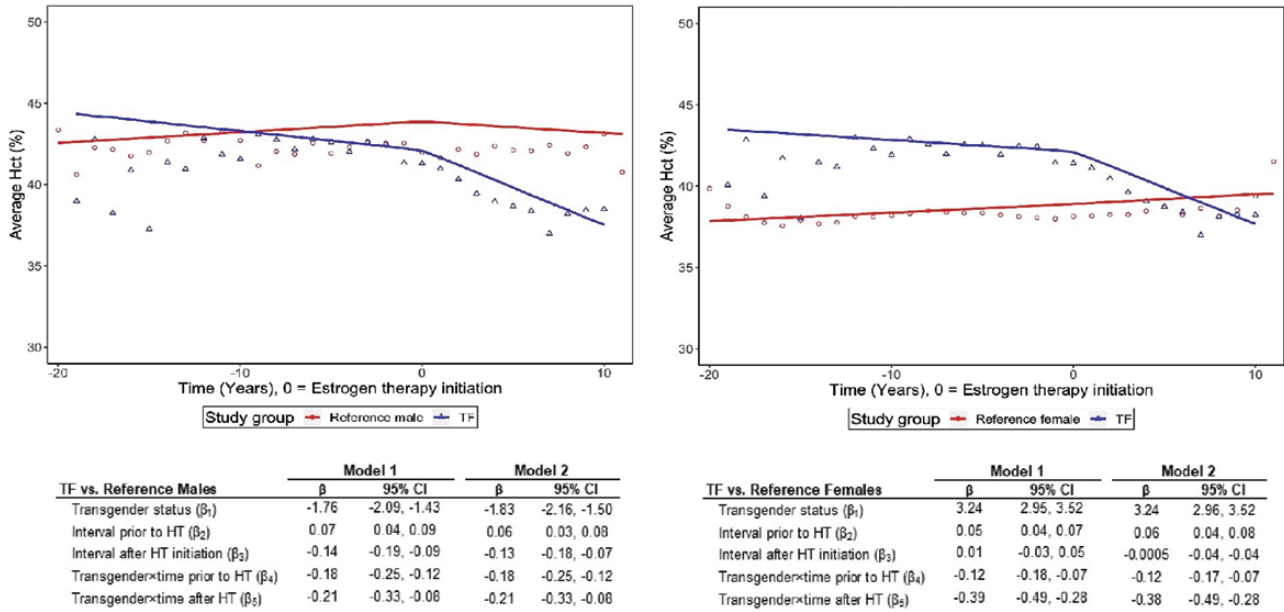
TF vs. Reference Males	Model 1		Model 2	
	β	95% CI	β	95% CI
Transgender status (β₁)	-1.76	-2.09, -1.43	-1.83	-2.16, -1.50
Interval prior to HT (β₂)	0.07	0.04, 0.09	0.06	0.03, 0.08
Interval after HT initiation (β₃)	-0.14	-0.19, -0.09	-0.13	-0.18, -0.07
Transgender×time prior to HT (β₄)	-0.18	-0.25, -0.12	-0.18	-0.25, -0.12
Transgender×time after HT (β₅)	-0.21	-0.33, -0.08	-0.21	-0.33, -0.08

TF vs. Reference Females	Model 1		Model 2	
	β	95% CI	β	95% CI
Transgender status (β₁)	3.24	2.95, 3.52	3.24	2.96, 3.52
Interval prior to HT (β₂)	0.05	0.04, 0.07	0.06	0.04, 0.08
Interval after HT initiation (β₃)	0.01	-0.03, 0.05	-0.0005	-0.04, -0.04
Transgender×time prior to HT (β₄)	-0.12	-0.18, -0.07	-0.12	-0.17, -0.07
Transgender×time after HT (β₅)	-0.39	-0.49, -0.28	-0.38	-0.49, -0.28

Figure 3. Predicted population average Hct values for TF individuals before and after HT initiation compared with matched referent males and females and estimates of linear mixed models for fixed effects.
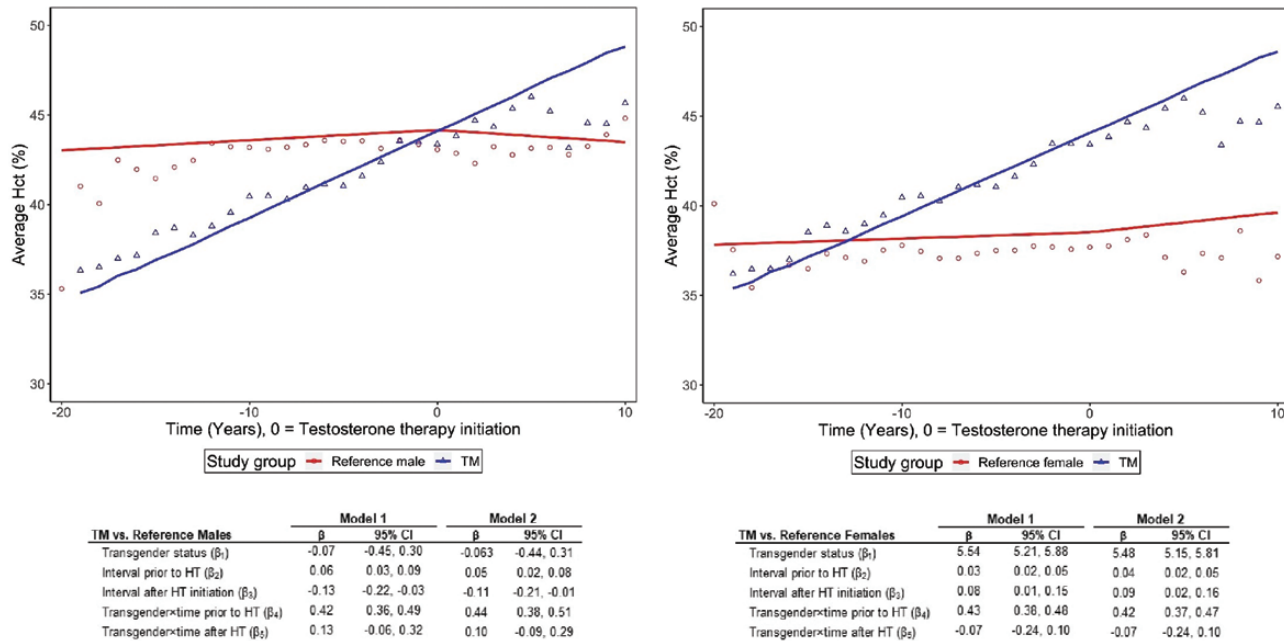

TM vs. Reference Males	Model 1		Model 2	
	β	95% CI	β	95% CI
Transgender status (β₁)	-0.07	-0.45, 0.30	-0.063	-0.44, 0.31
Interval prior to HT (β₂)	0.06	0.03, 0.09	0.05	0.02, 0.08
Interval after HT initiation (β₃)	-0.13	-0.22, -0.03	-0.11	-0.21, -0.01
Transgender×time prior to HT (β₄)	0.42	0.36, 0.49	0.44	0.38, 0.51
Transgender×time after HT (β₅)	0.13	-0.06, 0.32	0.10	-0.09, 0.29

TM vs. Reference Females	Model 1		Model 2	
	β	95% CI	β	95% CI
Transgender status (β₁)	5.54	5.21, 5.88	5.48	5.15, 5.81
Interval prior to HT (β₂)	0.03	0.02, 0.05	0.04	0.02, 0.05
Interval after HT initiation (β₃)	0.08	0.01, 0.15	0.09	0.02, 0.16
Transgender×time prior to HT (β₄)	0.43	0.38, 0.48	0.42	0.37, 0.47
Transgender×time after HT (β₅)	-0.07	-0.24, 0.10	-0.07	-0.24, 0.10

Figure 4. Predicted population average Hct values for TM individuals before and after HT initiation compared to matched referent males and females and estimates of linear mixed models for fixed effects.

estimates in that study were similar (39–51 for TM and 35–49 for TF) to those in our study (37–52 for TM and 34–48 for TF), and the 95% CIs largely overlapped. The authors concluded that the hematologic parameters for transgender men and women receiving HT "should be evaluated against the cisgender male and cisgender female reference ranges, respectively and does not require concurrent sex hormone analysis"[26].

Our results also indicate that the timing of changes in hematologic parameters in relation to HT initiation may differ among TM and TF study participants; whereas among TF cohort members, the downward change in the Hb and Hct trend coincided with the start of feminizing HT, the corresponding inflection point was not observed after the first filled prescription for testosterone among TM study participants. Rather, the data seem to indicate

Table 2. Incidence of Erythrocytosis in the Transgender and Matched Reference Cohorts[a]

Comparisons	Erythrocytosis Cases[a]	Rate (per 100 000 PD)	Hazard Ratio (95% CI)	
			Model 1[b]	Model 2[c]
TM	26	7.5	5.5 (3.3, 9.2)	7.4 (4.1, 13.4)
Reference males	17	1.4	1.0 (reference)	1.0 (reference)
TM	91	39.3	81.4 (41.1, 161.3)	83.1 (36.1, 191.2)
Reference females	6	0.39	1.0 (reference)	1.0 (reference)
TF	3	0.4	0.4 (0.1, 1.2)	0.4 (0.1, 1.1)
Reference males	40	1.1	1.0 (reference)	1.0 (reference)
TF	15	2.4	4.4 (2.4, 8.1)	1.0 (0.4, 2.5)
Reference females	23	0.5	1.0 (reference)	1.0 (reference)

Abbreviations: CI, confidence interval; PD, person-days; TF, transfeminine; TM, transmasculine.
[a]Defined as Hct > 52% and Hct > 48% in the analyses comparing transgender participants with reference cisgender males and cisgender females, respectively.
[b]Model 1: Inverse selection probability-weighted model (to account for eligible persons with no laboratory testing during follow-up) for transgender participants and referents matched on age, race/ethnicity, and study site; model stratified on cluster ID to account for matching.
[c]Model 2: Same as Model 1 and adjusted for the most recent Hct result before initiation of hormone therapy.

Table 3. Incidence of Anemia in the Transgender and Matched Reference Cohorts[a]

Comparisons	Anemia Cases[a]	Rate (per 100 000 PD)	Hazard Ratio (95% CI)	
			Model 1[b]	Model 2[c]
TM	22	28.6	2.2 (1.3, 3.7)	3.1 (1.8, 5.6)
Reference males	27	13.8	1.0 (reference)	1.0 (reference)
TM	13	4.8	0.2 (0.1, 0.3)	0.3 (0.1, 0.4)
Reference females	181	26.4	1.0 (reference)	1.0 (reference)
TF	160	96.7	6.0 (4.7, 7.6)	5.9 (4.6, 7.5)
Reference males	138	15.0	1.0 (reference)	1.0 (reference)
TF	92	16.3	0.9 (0.7, 1.1)	1.4 (1.1, 1.8)
Reference females	357	16.9	1.0 (reference)	1.0 (reference)

Abbreviations: CI, confidence interval; PD, person-days; TF, transfeminine; TM, transmasculine.
[a]Defined as Hct < 42% and Hct < 36% in the analyses comparing transgender participants with reference cisgender males and cisgender females, respectively.
[b]Model 1: Inverse selection probability-weighted model (to account for eligible persons with no laboratory testing during follow-up) for transgender participants and referents matched on age, race/ethnicity, and study site; model stratified on cluster ID to account for matching.
[c]Model 2: Same as Model 1 and adjusted for the most recent Hct result prior to initiation of hormone therapy.

that TM subjects experienced a continuous increase in Hb and Hct for a number of years before the start of their HT. The reason for this observation is not entirely clear. It is possible that some of the transgender cohort members used cross-sex hormones before they began receiving HT at Kaiser Permanente, although it seems unlikely that this would occur only in TM but not in TF participants, as previous studies have shown that unsupervised hormone use is common in both groups [30, 31]. Other possible explanations, such as smoking or history of amenorrhea or hypomenorrhea in TM individuals before testosterone initiation, also warrant consideration.

The literature on longitudinal changes in hematology parameters following HT is relatively sparse. One recent study examined data pertaining to 265 TM and 340 TF individuals enrolled in the European Network for the Investigation of Gender Incongruence (ENIGI) cohort [21].

The TM participants were treated with injections of intramuscular testosterone esters or testosterone undecanoate, or testosterone gel. The TF cohort members received cyproterone acetate in combination with either oral estradiol valerate or transdermal estradiol, depending on age. The participants were followed for 36 months and examined every 3 months. After 12 months of follow-up, the average Hct level of TM subjects increased from an average of 41% to 46% and remained relatively unchanged thereafter. The corresponding values for TF decreased from approximately 45% to 41% at 3 months and then stabilized.

Another study enrolled 101 adolescents and young adults (ages 12–24 years old) who presented with gender dysphoria to a specialized center at the Children's Hospital in Los Angeles [18]. The HT protocol in TF youth included a testosterone blocking agent in combination with oral or injectable 17-β estradiol. Transmasculine patients were

all treated with subcutaneous injections of testosterone cypionate. Fifty-six study participants (34 TM and 22 TF) had Hb levels measured at baseline and at 24 months of follow-up. Among TF subjects, the average Hb concentration decreased from 15.3 to 14.1 g/dL, whereas among TM subjects the average Hb level increased from 13.0 to 15.5 g/dl. According to the authors, one participant developed borderline anemia, but the remaining results were within the normal range for cisgender females.

Several mechanisms have been proposed to explain the erythropoietic effect of testosterone, including the direct stimulation of bone marrow progenitors, stimulation of erythropoietin and changes in the physiological erythropoietin set point for a given Hb level, and an increase in iron availability and utilization by suppression of hepcidin transcription [32–35]. In some cases, this may lead to erythrocytosis, although clinical implications of this phenomenon, when it occurs secondary to testosterone therapy, are not clear.

In the previously cited ENIGI study, 22/192 TM participants receiving testosterone developed erythrocytosis based on the Hct cutoff of >50% and 4 cohort members met the criteria for erythrocytosis using the cutoff of >52% [21]. In our study, 26/398 (7%) TM participants with no prior evidence of "male" erythrocytosis (defined as Hct over 52% for males) at the start of HT experienced Hct elevation above that cutoff. Similarly, 91/311 (29%) participants who never met the criteria for female erythrocytosis crossed the Hct threshold of 48% during follow-up.

According to the Endocrine Society guidelines, clinically significant testosterone-induced erythrocytosis can be treated with therapeutic phlebotomy; however, there is no clear Hct cutoff above which therapeutic phlebotomy is clearly indicated [36]. If clinically significant erythrocytosis is a concern, lowering the testosterone dose or switching to transdermal testosterone administration, which at its typical starting regimen delivers a lower amount of hormone than the injectable formulations, may offer a reasonable alternative because increases in Hct and Hb may be related to testosterone dose [37, 38].

A distinguishing feature of the STRONG cohort is the systematic identification of eligible participants enrolled in large community-based health plans. The use of EHR data ensures that all eligible individuals are included in the analyses, as participation does not require subject opt-in. The well-defined source population also allows selecting matched cisgender comparators who have the same demographic characteristics and reside in the same geographic areas. Another methodological advantage of our study is the relatively long follow-up and the use of repeated measures that enabled the assessment of trajectories of the changes in Hb and Hct.

The observational EHR-based design of this study comes with limitations. Unlike clinic-based cohorts, such as ENIGI, the bloodwork for STRONG was not collected at predetermined intervals specifically for the purpose of the study, and the number and frequency of sample collection differed across participants. We attempted to address this issue, at least in part, by performing weighted analyses, but more definitive results could have been obtained if all eligible participants underwent testing of the same frequency and duration. Whereas the information on HT received within the Kaiser Permanente system has been shown to be accurate [24], the lack of information on HT outside the Kaiser Permanente is a potential limitation. As mentioned previously, this restricts our ability to identify a subcategory of cohort members who were truly hormone-naïve at the start of follow-up. We attempted to address this issue by focusing on STRONG participants whose EHR demonstrated a gap between the index date and the first filled HT prescription, but it is possible that at least some of the individuals enrolled in the "hormone initiation" subcohort may have already received hormones at the start of follow-up.

A more definitive identification of hormone-naïve transgender persons at the start of HT can be based on hormone levels; these data are now increasingly available in the EHR and will be the focus of future analyses. Additional analyses are also needed to better understand the clinical significance of Hb and Hct changes and to examine patterns of longitudinal changes in these and other laboratory markers in relation to receipt of gender-affirming gonadectomy, and according to changes in HT use, formulations and doses.

The World Professional Association for Transgender Health and the Endocrine Society recommend monitoring a variety of laboratory values in transgender persons receiving HT [39, 40]. The present study offers new longitudinal data that, in addition to previous reports [17–19, 21], may inform the development of much-needed reference ranges specific to this sizeable and understudied and underserved population. Our results support the recommendation [26] that hematological parameters of transgender people receiving HT should be interpreted based on their affirmed gender, rather than their sex documented at birth. The clinical significance of erythrocytosis following testosterone therapy, as well as anemia following feminizing HT, requires further investigation.

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Additional Information

Correspondence: Michael Goodman, MD, MPH, Department of Epidemiology, Emory University School of Public Health, 1518 Clifton Road, NE, Atlanta, GA 30322, USA. E-mail: Mgoodm2@emory.edu.

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