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Review

When Gender Identity Doesn't Equal Sex Recorded at Birth: The Role of the Laboratory in Providing Effective Healthcare to the Transgender Community

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BACKGROUND: Transgender is an umbrella term used to describe individuals who identify with a gender incongruent to or variant from their sex recorded at birth. Affirming gender identity through a variety of social, medical, and surgical interventions is critical to the mental health of transgender individuals. In recent years, awareness surrounding transgender identities has increased, which has highlighted the health disparities that parallel this demographic. These disparities are reflected in the experience of transgender patients and their providers when seeking clinical laboratory services.

CONTENT: Little is known about the effect of gender-affirming hormone therapy and surgery on optimal laboratory test interpretation. Efforts to diminish health disparities encountered by transgender individuals and their providers can be accomplished by increasing social and clinical awareness regarding sex/gender incongruence and gaining insight into the physiological manifestations and laboratory interpretations of gender-affirming strategies. This review summarizes knowledge required to understand transgender healthcare including current clinical interventions for gender dysphoria. Particular attention is paid to the subsequent impact of these interventions on laboratory test utilization and interpretation. Common nomenclature and system barriers are also discussed.

SUMMARY: Understanding gender incongruence, the clinical changes associated with gender transition, and systemic barriers that maintain a gender/sex binary are key to providing adequate healthcare to transgender community. Transgender appropriate reference interval studies are virtually absent within the medical literature and should be explored. The laboratory has an important role in improving the physiological understanding, electronic medical system recognition, and overall social awareness of the transgender community.

The transgender population challenges social norms because it personifies the differences between biological sex and gender. Biological sex, defined by anatomical characteristics and chromosomal makeup, can be the same as or different from a person's gender identity, which is defined as a person's psychological identification as male, female, or nonbinary. The word transgender is used to describe an individual whose gender identity is different or incongruent from the sex assigned at birth (Table 1).

Transgender identity or gender incongruence is not a disease. Gender dysphoria is a psychological syndrome or pathology related to the distress an individual can experience when their gender is incongruent and is thought to be a primary reason for the high rates of transgender-related suicides and suicide attempts *(1)*. Not all transgender individuals experience gender dysphoria, but one goal of transitioning, or using social and medical procedures to affirm gender identity, is to relieve the person of this distress. In the absence of any gender dysphoria, gender-affirming medical interventions are used to align physical appearance with gender identity. The transition process for some individuals may include physical elements (hormone replacement therapy, surgeries), but for others transitioning socially (without hormones/surgeries) meets their needs *(2)*.

The laboratory has medically significant reasons for classifying samples based on biological sex, which are both clinical and pragmatic. For example, calculation of estimated glomerular filtration rate (eGFR)[4] requires the

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[4] Nonstandard abbreviations: eGFR, estimated glomerular filtration rate; PSA, prostate-specific antigen; hCG, human chorionic gonadotropin; CBC, complete blood count; FSH, follicle stimulating hormone; LH, luteinizing hormone; EMR, electronic medical record; LIS, laboratory information system; LC-MS/MS, liquid chromatography–tandem mass spectrometry; IA, immunoassay.

Table 1. Common transgender-related nomenclature.

Term	Definition
Birth sex	Sex assigned and recorded based on the visual appearance of sex organs or by karyotype in ambiguous cases
Cisgender	Congruence between birth sex and gender identity
Disorders of sexual development	Reproductive differences caused by genetic mutations. Includes intersexed individuals. Not a common etiology for transgender men or women
Female to male; transman	Recorded as female at birth; gender identity male
Gender	Inherent identity as male or female based on categories that are socially and culturally constructed
Gender dysphoria	Moderate to severe anxiety, depression, and/or distress that results from internal sense of discordance and/or negative external experience living within sex-concordant socially defined gender contexts and constructs
Legal sex	Sex as defined by legal documents. Can be congruent with either birth sex or gender identity
Male to female; transwoman	Recorded as male at birth; gender identity female
Nonbinary	Having a gender associated with neither male/female or inherently identifies as both male and female
Pronoun	A word used to describe a person that is often gender specific
Sexual orientation	The desire to have sexual relations with someone of the same or different gender identity and/or anatomical sex
Transsexual	A term in the English language which has been used historically to pathologize gender variance and incongruence
Transgender	An umbrella term used to describe individuals who identify with a gender incongruent or variant with their sex recorded at birth
Transition	The medical and social process a transgender person may undertake to facilitate congruency between their gender identity and their socially perceived gender

input of sex to accurately assess kidney function *(3–5)*. Similarly, healthy hematologic parameters are significantly different between cisgender (Table 1) men and women *(6–8)*. Finally, biomarkers assumed to be sex specific, such as prostate-specific antigen (PSA) and human chorionic gonadotropin (hCG), are often overlooked if ordered on the "incorrect" sex *(9)*. Where does the transgender population medically fit into these sex-based categories? How can a physician adequately assess anemia in an individual who has transitioned from one gender to another–especially considering that the goal of an individual's transition is not defined by a medical procedure or therapy, but by a psychological ease that is person-specific? This review provides an overview of the important aspects to consider in regard to transgender healthcare, with an emphasis on laboratory's role.

Epidemiology

Until recently, the estimated prevalence of transgender or gender-nonconforming persons was based primarily on those who presented to physicians with symptoms related to gender dysphoria, or those seeking gender transition from specialist gender clinics *(10)*. This narrow case definition informed a collective and historic Western understanding that the transgender state was extremely rare *(11)*. Work over the last decade has revealed that these historic numbers grossly underestimated the size of the broader transgender population, which includes a spectrum of gender identity and incongruence. This was in large part due to barriers that existed, and arguably still exist, for transgender persons trying to access knowledgeable, relevant, and supportive care. These barriers are well described by the concept of active and passive erasure: informational systems and institutional policies and practices that do not acknowledge the existence of transgender persons and in some cases perpetuate systemic transphobia *(12)*. Examples of barriers include the following: lack of knowledgeable care providers, little or no medical research related to the health of transgender persons, a focus on being transgender when receiving clinical care for a problem unrelated to gender incongruence, and the inability to be represented in an electronic health record as neither male or female. More direct methods for estimating population sizes, in which samples from the general population were questioned about their identity, generated estimates ranging from 0.5% to 1.3% for

Table 2. Surgical interventions for transgender people.

Procedure	Common recipient	Intended effects
Vaginoplasty	Transgender women	Create a vagina and vulva that are fully functional from a sexual and aesthetic perspective
Phalloplasty	Transgender men	Create a functional penis from a sexual and aesthetic perspective; may or may not include functional neourethra
Metoidoplasty	Transgender men	Create a small penis from hypertrophied clitoris; may or may not include functional neourethra
Hysterectomy and salpingo-ooverectomy	Transgender men	Removal of uterus and ovaries to eliminate most estrogen production; prevents health complications, treats unresolved menstrual cramping; induces further masculinization
Orchiectomy/orchidectomy	Transgender women	Removal of testes to eliminate androgen production; induces further feminization
Male chest reconstruction	Transgender men	Modified mastectomy to create a more male-appearing chest
Female chest reconstruction	Transgender women	Ranges from simple breast augmentation to more involved reconstructive work with augmentation
Facial feminization	Transgender women	Refers to a series of procedures that reshape traditionally masculine appearing features into more feminine features, i.e., tracheal shave or brow reconstruction

birth-assigned males, and from 0.4% to 1.2% for birth-assigned females *(13)*.

Etiology

Early efforts to understand gender incongruence date back to the late 1800s, and were firmly rooted in the psychopathologization. Psychoanalytical and environmental theories dominated the literature well into the 1990s and informed most clinical approaches to the management of gender variance and, when present, the associated dysphoria *(14)*. Many of these theories were based on the postulate that individuals were psychosexually neutral at birth, with gender identity evolving and consolidating solely based on postnatal social interaction and experiences. This perspective changed dramatically with a number of seminal case series and retrospective studies describing iatrogenic gender dysphoria in persons with differences in sexual development who were reassigned to a gender that was discordant from their original anatomical and genetic sex recorded at birth. This introduced the concept of gender identity as determined, at least in part, before or during embryonic and fetal development *(15)*. A range of influences including genetic, hormonal, and neuroanatomical factors are now thought to be the main contributors in the development of a person's gender identity *(15)*.

In 2010, a consensus statement by the World Professional Association for Transgender Health, whose membership includes specialists in the management of gender incongruence and related dysphoria across numerous cultures, emphasized the importance of the depsychopathologization of the transgender individual *(16)*. This included a major shift away from the "why" toward the "how." Care of transgender persons began to focus on supporting the social and emotional aspects related to being gender variant, and on medical solutions to resolve the discordance *(17)*.

Clinical Management

Transgender people often seek medical and surgical intervention to align their external primary and secondary sex characteristics with their internal gender identity. The goal of therapy is to align gender identity with gender expression and/or to reduce the distress caused by gender dysphoria. Surgical intervention focuses on the reconstruction of chests, faces, and genitals to be more in line with the appearance of a patient's gender identity (Table 2) *(18)*. Endocrine intervention allows the development of secondary sex characteristics ranging from changes in hair pattern to increases or decreases in chest size *(19)*.

Guidelines exist for treating and monitoring transgender patients receiving hormone therapy, but they are largely based on expert opinion *(20–22)*. There are very few evidence-base studies to guide clinicians in hormone management of transgender people. However, existing data do show clinically significant health benefits to aligning gender identity and expression with medical and surgical intervention *(1)*.

Transwomen are commonly treated with some form of estrogen with or without a testosterone blocker *(21)*. Estrogens are most commonly 17-β estradiol in oral,

Table 3. Clinical assessment of transgender people prescribed hormone therapy.

Analyte	Transwomen	Transmen
Testosterone	Goal: suppress testosterone to within reference intervals for a cisgender female	Goal: maintain total testosterone within reference intervals for a cisgender male
	Considerations: concentrations should be suppressed within 3 months of starting combined estrogen/antiandrogen therapy	Considerations: wide variation with injected testosterone regimens based on time since most recent dose
Estrogens	Goal: correlate estrogen concentration with estrogen dose	Goal: monitor for suppression of cyclical changes in concentration and overall decrease in concentration with initiation of testosterone therapy
	Considerations: there is large variation with injected doses, and no consensus on most effective use of monitoring estrogen concentration	Considerations: testosterone can be aromatized into estradiol, which indicates testosterone dose should be lowered
FSH	Goal: suppressed FSH as a surrogate indication of gonadotropin-releasing hormone production	Goal: differentiate between endogenous estrogen production and aromatization of testosterone
	Considerations: indicates that testes are not producing testosterone; inadequate suppression is an indication to increase estrogen or antiandrogen dose	Considerations: increased FSH indicates endogenous production of estrogens
Prolactin	Goal: monitor for pituitary microadenoma enlargement secondary to estrogen administration	Goal: monitor for pituitary microadenoma enlargement secondary to aromatized estrogen
	Considerations: microadenoma growth is caused by estrogen and is an uncommon side effect of therapy; lesions are most frequently subcentimeter in size and of little clinical significance	Considerations: microadenoma growth is caused by estrogen, and is an uncommon side effect of therapy; lesions are most frequently subcentimeter in size and of little clinical significance

transdermal, or injectable formulations. Other estrogens, such as conjugated equine estrogens, are also used; however, the risk of each estrogen derivative should be assessed for its thrombogenic risk, which should be discussed with the individual *(23–25)*. Spirolactones are the most commonly prescribed testosterone blockers, but 5-α reductase inhibitors and gonadotropin releasing hormone agonists are also used depending on age and tolerability *(20, 26)*.

Goals of estrogen therapy vary from patient to patient; however, breast development, subcutaneous fat redistribution, and skin softening are commonly desired *(2)*. Patients often report wishing to look and feel "more feminine" without any specific body changes. The hormone regimen a patient uses should be tailored to meet their goals, including questions around sexual function and fertility preservation, as blocking testicular function will inhibit both *(20)*. Combined estrogen/testosterone blocker therapy will also cause testicular and penile atrophy *(27)*. Educating patients about the effects, and also limitations, of hormone therapy is crucial to management.

The primary limitations of estrogen therapy are the associated interindividual variability and the lack of clinical studies elucidating the reasons for the variable effects. Transwomen on the same hormone regimen may have drastically different response to treatment, and there is currently no validated measure to predict the degree of change *(22)*. Studies dissecting how systemic hormone concentrations relate to hormone dose and subsequent phenotypic presentation are critical. Hormone therapy will not affect primary sex characteristics. These limitations are the reason surgical interventions are often medically necessary *(18)*. Surgical interventions for transwomen include facial, chest, and genital reconstruction (Table 2).

In the treatment of transmen, patients most often receive testosterone alone (without estrogen suppression) either transdermally or intramuscularly *(27)*. In some cases, estrogen suppression can be necessary to inhibit menstruation or minimize monthly menstrual cramping that occurs with amenorrhea, or because of more rare complications to treatment *(20, 22)*. Patients often initiate therapy seeking facial and body hair growth, cessation of menses, subcutaneous fat redistribution, voice change, and clitoromegaly *(2)*. Side effects of testosterone therapy include male pattern baldness, infertility, and vaginal dryness *(27)*.

Much like estrogen therapy for transwomen, testosterone therapy for transmen does not affect primary sex characteristics. Mammary tissue in a transgender man's chest is also unaffected and as such, creating a male appearing chest through surgical mastectomy is common. Similarly, surgical construction of male genitals is sometimes pursued (Table 2) *(18)*.

For pediatric patients, gender incongruence therapies depend on the Tanner stage and, if present, degree of gender dysphoria. The American Academy of Pediatrics has released a guideline document to assist practitioners caring for transgender youth *(28)*. In brief, the social transition of the child should be supported with respectful pronouns, preferred name, and gender expression. Gonadotropin-releasing hormone agonists are recommended to delay puberty, and cross-sex hormone therapy may be indicated at a developmentally appropriate age *(26)*.

Empirical Use of the Laboratory to Clinically Monitor Transgender People

Not all transgender people choose to initiate hormone therapy, but for those who do there are several laboratory tests that are perceived to be clinically useful for monitoring the safety and progress of hormone therapy *(22)*. Baseline and follow-up chemistry panels help screen for potential increase in transaminase activity and electrolyte imbalances. Transwomen often receive spironolactone to suppress testosterone production, and it is particularly important to monitor potassium concentration when this medication is prescribed *(29)*. Both estrogens and testosterones have been reported to cause increases in liver enzyme activities, but no published data suggest significant incidence rates or pathological relevance *(25, 30, 31)*.

Testosterone and estrogen therapies affect hemoglobin and hematocrit *(24, 25, 30–32)*. There is debate among clinicians as to the correct reference interval for transgender men and women compared to their cisgender counterparts, and little data to suggest proper reference intervals among transgender people. Questions about the treatment of increased or decreased hemoglobin and hematocrit are very common among primary care providers consulting with transgender care specialists. The most common empirical assumption is that the complete blood count (CBC) results should parallel the hormone profile. This means that transmen prescribed testosterone for >6 months should have their values compared to the cismale reference interval, and transwomen prescribed estrogen for >6 months should have their values compared to the female reference interval. Some experts suggest that transgender women should be evaluated for anemia when their hemoglobin is below the lower limit of the cisfemale reference interval. Workup should be evaluated based on size and chromaticity of red blood cells. For transgender men, monitoring hyperchromic polycythemia secondary to testosterone use is important because androgens stimulate erythropoiesis. Treatment with therapeutic blood draw or decreasing testosterone dose should be considered when hemoglobin values are consistently above the upper limit of the cismale reference interval. Anemia in transgender men on testosterone is uncommon and should be evaluated and addressed in the same manner as anemia in cisgender men.

Hormone supplementation can influence glucose and lipid metabolism *(30)*. Estrogen therapy in transwomen can be associated with weight gain and increased HDL. Triglyceride concentrations are important to monitor, especially in the context of transmen on medication that increases the risk for pancreatitis *(25, 31)*, such as nucleotide/nucleoside reverse transcriptase inhibitor therapy for the treatment of the human immunodeficiency virus *(32)*. Testosterone supplementation in transmen can increase LDL. There is some speculation connecting insulin resistance, metabolic syndrome, and hormonal treatment, but this research is still in its infancy *(33)*. Both testosterone and estrogen therapy are associated with weight gain, but not to a degree that justifies more than routine diabetes screening. Hemoglobin A1c (glycohemoglobin; or fasting glucose) and lipid parameters should be evaluated in accordance with current population guidelines.

Sex hormones are often monitored to help understand the individual patient's response to hormone therapy *(20–22)*. Testosterone and estradiol concentrations are useful to guide hormone therapy dose. For transmen, testosterone concentration should be within the reference interval established for cisgender men. Testosterone therapy can be prescribed for transdermal or intramuscular use. If intramuscular is prescribed the observed testosterone concentration will be influenced by timing of the previous dose until steady state is reached. Ideally, testosterone concentrations do not exceed the upper limit of the reference interval for a cisgender male even at peak dose and are not below the lower limit of the cisgender male reference interval at trough.

When prescribing estradiol and testosterone blockers to transwomen, testosterone concentrations should be evaluated against the ciswomen reference interval *(20–22)*. Testosterone is often suppressed to undetectable concentrations. This is not a problem unless the patient is symptomatic for low testosterone (lethargy, decreased libido) *(34)*. Symptoms are usually resolved by discontinuing testosterone suppression therapy or by adding low-dose testosterone (for those who are postgonadectomy). Without testosterone suppression during estrogen therapy, testosterone concentrations are often slightly below the lower limit of the reference interval for cismen. This

concentration allows the patient to retain some erectile function, which is sometimes a goal.

Estradiol and progesterone concentrations are often monitored, but their utility is not yet understood *(21, 22)*. No therapeutic ranges have been defined empirically for those in transition. Symptoms and signs of a therapeutic concentration include breast tenderness, breast growth, and adipose redistribution during transition from male to female. There is no evidence thus far that serum concentrations offer any advantage to clinicians or patients over and above the historical and physical estrogenic impact measured over time. The literature remains silent on an appropriate posttransition maintenance serum concentration or dose, beyond the lowest possible dose to avoid potential side effects from estrogenic hormone supplementation.

Follicle stimulating hormone (FSH) and luteinizing hormone (LH) are both useful in determining the efficacy of hormone therapy and how to make hormone therapy more effective for an individual patient. Estrogen therapy should cause FSH suppression, and therefore FSH should be evaluated after initiation of hormones and annually to assess hormone efficacy *(25)*. Testosterone therapy will also cause FSH depression *(35)*. Evaluating FSH in parallel to estrogen is important because increased FSH indicates endogenous estrogen production, as opposed to aromatization of testosterone when FSH is suppressed. Postgonadectomy transmen prescribed testosterone replacement therapy will have highly increased FSH concentrations that are comparable to posthysterectomy or postmenopausal cisgender women. Increased LH concentrations correlate with a loss in bone density among transmen in the first year of testosterone therapy, which is likely a function of the fluctuating sex steroid profile *(36)*.

Prolactin concentrations should be measured annually in transwomen on estrogen therapy as estrogen can cause preexisting pituitary adenomas to grow and increase prolactin secretion *(25)*.

System Barriers to Transgender Care

Barriers to healthcare, both front-of-the-house and within the laboratory, prevent an overwhelming majority of transgender-identified individuals from accessing basic medical care *(9, 37, 38)*. Most transgender individuals have, at some point in their lives, avoided seeking medical treatment because of their fears of discrimination. Survey data shows consistent self-reporting of healthcare avoidance (25%–30%), refusal of care (15%–20%), and harassment or violence in a healthcare setting (25%–30%) *(39, 40)*. The laboratory contributes to these statistics *(9)*. System barriers imposed by the laboratory can be divided into 3 categories: electronic medical systems and interfacing limitations, sample collection, and generalized rules that cancel or improperly flag results based on sex.

Most electronic medical systems, including the electronic medical record (EMR) and laboratory information systems (LIS), have limited capabilities to extend beyond a gender or sex binary, which can have medical and emotional consequences for patients *(41)*. In October 2015, following recommendations by the Institute of Medicine and the Joint Commission, CMS (Centers for Medicare and Medicaid Services) and the Office of National Coordinator for Health Information Technology added sexual orientation and gender identity to the list of required fields for electronic health software to be considered certified for Meaningful Use *(42, 43)*. These guidelines portend a need for laboratory results that can be flagged based on gender or sex, as appropriate. In fact, the May 2016 release of section 1557 of the Affordable Care Act (the document outlining Nondiscrimination in Health Programs) outlines specific guidelines for the laboratory to be educated to transgender healthcare needs. The laboratory is underprepared for adoption of these principles, but should nevertheless begin to integrate the reasoning into their system designs. The first step will be to integrate gender identity fields into the institutional EMR *(44, 45)*. A web-based tool designed and maintained by University of California San Francisco can facilitate this step *(45)*. The next 2 steps should be completed in parallel, first to integrate similar gender identity descriptions into LIS fields, and second to collect data pertaining to normal ranges associated with transgender individuals. The latter will be further described in later sections of this review.

A primary role of the phlebotomist is to confirm that the identity listed on the barcode matches the identity of the patient having their sample collected. In most institutions, phlebotomy is a component of the laboratory and therefore the laboratory director is responsible for proper quality management. Diversity training is not commonly required for phlebotomists, which leaves them vulnerable to social ignorance, including a lack of transgender awareness. Given the absence of specific identifiers for gender within most EMR/LIS systems, a visual sex/gender discrepancy can cause confusion leading to sample processing delays, mislabels, lost samples, or other preanalytical errors. The solution to this risk is ensuring the phlebotomy staff is sensitive to gender identity and implementing gender-sensitive procedures for the phlebotomy staff to reference as needed.

The final system barrier imposed onto the transgender community by the laboratory is the seemingly intuitive nature of canceling or improperly flagging results because they "should be" sex specific. For example, some institutions cancel pregnancy tests ordered on male patients. These rules are often established to avoid insurance fraud or laboratory confusion but can be harmful to transgender patients. Transmen may maintain their reproductive capabilities and can have a sexual orientation

compatible with fertilization *(46, 47)*. Performing the hCG assay as ordered ensures that these tests provide the results necessary for clinical evaluation. A second example is regarding PSA screening. Author D.N. Greene was consulted on a case for which a missed cancer diagnosis occurred because PSA reference intervals, and hence flagging, were not appended to female samples in a large national reference laboratory. The test had been properly ordered for screening in this patient, but values of 11 and 15 ng/mL went unnoticed (due to lack of flagging) until the PSA concentration was >100 ng/mL and the prostate cancer was stage III *(48)*. PSA values >4 ng/mL should be flagged regardless of the sex listed in the electronic health record.

Discussion of Sex-Specific Reference Intervals and the Transgender Population

Establishing proper reference intervals is a complicated and evolving branch of clinical chemistry *(49–51)*. Population partitioning is clinically indicated, but imperfect, and often imposes overarching generalizations. Age, sex, and racial demographics are the most common measures that divide populations. Establishment of sex-specific reference intervals based on an assumed binary defined by chromosomal makeup is practical and usually provides the information necessary to diagnose and monitor cisgender patients. Sex-specific reference intervals are commonplace for several critical analytes and can be divided into hematology, general chemistry, and special chemistry tests *(8, 50, 52)*. By contrast, reference interval studies specific to the transgender population are strikingly absent within the literature *(9)*. There are, however, select publications that look at the influence of hormone therapy on these analytes (Table 4) *(25, 31, 33, 35, 53–56)*. These studies do not correlate laboratory values to clinical phenotype and instead just look for global changes in concentration from baseline after initiation of hormone therapy.

CBCs are an indispensable component to primary care and display considerable differences between the sexes *(6, 8)*. The onset of puberty triggers hematologic parameter differences in the sexes that persist into old age *(8)*. Red cell count, hematocrit, and hemoglobin increase at puberty for males, but not females. In contrast, platelet counts decrease in both sexes at puberty, but significantly more in men compared to women *(8, 57)*. As such, sex-specific reference intervals are generally applied to these hematologic parameters around age 14. Little has been published about what defines "normal" in a transgender individual. Roberts et al. showed that compared to matched cisgender individuals, hematocrit and hemoglobin concentrations in transwomen more similarly resembled those in ciswomen *(31)*. Additional studies evaluated the change in laboratory values from baseline in transmen and saw statistically significant increases in hemoglobin, hematocrit, and red cell count *(25, 53, 55)*. One study evaluated hematocrit changes after estrogen therapy in transwomen and saw a statistically significant decrease *(25)*. These studies did not provide reference intervals for comparison to the pre- or posthormone values, but erythrocytosis was noted for 2 transmen.

Sex-specific reference intervals for general chemistry analytes are often a function of tissue mass between males and females, with males having larger organs and therefore higher baseline concentrations of tissue-specific markers and metabolic products. In general, men will have higher numerical reference interval for enzymes, especially those related to cardiac, liver, and muscle tissue turnover *(50)*. Liver enzyme increases have been documented in both transmen and transwomen, but some studies show that supplementation with testosterone will increase liver enzymes relative to baseline, while estrogen will cause a decrease (Table 4) *(25)*. Other studies show that liver enzymes do not significantly change *(31, 53, 55)*. Metabolic products follow a similar trend, with creatinine, uric acid, and urea having higher concentration ranges for males *(50)*. While all of these metabolites are largely unstudied in the transgender population, there are indications that creatinine values will decrease in transwomen and increase in transmen *(31)*; there is no empirical or published evidence of which eGFR equation is better suited for transgender individuals.

Lipid profiles between sexes are interesting, with cismen showing higher LDL and triglyceride concentrations, while ciswomen have higher HDL concentrations *(50)*. As expected, this parallels a higher apolipoprotein B in cismen and higher apolipoprotein A1 in ciswomen. Decision points for basic lipid panels are outcome based and set equivalent between sexes, but if combined with other assessments of cardiac risk can be complicated by sex-specific variations in homocystine and high-sensitivity C-reactive protein *(52)*. Several studies have shown that in transmen LDL is increased and HDL decreased compared to baseline concentrations, while transwomen showed a decreased LDL and increased HDL, but other studies showed no change in these parameters or the inverse effect in transwomen (Table 4) *(25, 31, 33, 35, 53, 55, 56)*. Similarly, these studies show that triglyceride concentrations either increase or are unchanged in transmen; publications document overall increases, decreases, and no change in triglyceride concentration in transwomen.

Laboratory testing outside the basic or comprehensive metabolic assessment can also be influenced by sex. As described earlier, many hematologic parameters are influenced by sex and therefore it is unsurprising that iron supply markers would also show sex-specific variation *(52)*. Ferritin is relatively higher in cismen; total iron concentration is lower in ciswomen. There are no studies that evaluate iron markers in transgender individuals.

Table 4. Published observational changes in laboratory values for transgender people on hormone therapy irrespective of phenotypic response.

	Transwomen	Transmen
	Hematology	
Increased		Hematocrit *(25, 35, 53, 55)*[a]
		Hemoglobin *(35, 53, 55)*
		Red cell count *(53)*
Decreased	Hematocrit *(25, 31)*	
	Hemoglobin *(31)*	
	General chemistry	
Increased	HDL *(53, 56)*	ALT[b] *(25, 35)*
	LDL *(33)*	AST *(25, 35)*
	Total cholesterol *(33)*	Creatinine *(25, 53)*
	Triglycerides *(31, 33)*	Total cholesterol *(25, 33, 35)*
		Triglycerides *(25, 33, 35)*
Decreased	ALT (25)	HDL *(25, 33, 35, 55, 56)*
	AST *(25)*	
	Creatinine *(25, 53)*	
	HDL *(25, 33)*	
	LDL *(25, 31)*	
	Total cholesterol *(25)*	
	Triglycerides *(25)*	
No change	Total cholesterol *(31, 53, 56)*	LDL *(53, 56)*
	Triglycerides *(53, 55, 56)*	HDL *(53)*
		Total cholesterol *(53, 55, 56)*
		Triglycerides *(25, 53)*
	Endocrine/special chemistry	
Increased	Estrogen *(25)*	Testosterone *(25, 35, 54)*
	Prolactin *(25)*	FSH *(54)*
	SHBG *(25)*	DHEAS *(54)*
Decreased	Testosterone *(25)*	Estrogen *(25, 35)*
	FSH *(25)*	SHBG *(25, 35, 54)*
	LH *(25)*	AMH *(54)*
	DHEAS *(25)*	FSH *(25, 35)*
		LH *(25, 35)*
		Prolactin *(25, 35)*
		DHEAS *(35)*
No change		Estrogen *(54)*
		LH *(54)*
		DHEAS *(25)*

[a] References: Wierckx et al. *(25)*, Roberts et al. *(31)*, Mueller et al. *(35)*, Fernandez and Tannock *(53)*, Caanen et al. *(54)*, Pelusi et al. *(55)*, and Deutsch et al. *(56)*.
[b] ALT, alanine aminotransferase; AST, aspartate aminotransferase; SHBG, sex hormone binding; DHEAS, dehydroepiandrosterone.

In addition to estrogen, testosterone, progesterone, and their associated metabolites, other reproductive and pituitary endocrine markers have sex-specific ranges, including the following: FSH, LH, sex hormone binding globulin, antimullerian hormone, and prolactin. In transmen testosterone will increase with a concurrent decrease in estrogen; in transwomen estrogen will increase with a concurrent decrease in testosterone *(25, 35, 54)*. The gonadotropins LH and FSH were shown to decrease from baseline in 2 of these studies for transmen and transwomen, but 1 study showed no change in LH and an increase in FSH for transmen. Prolactin increases in transwomen and decreases in transmen *(35)*. Most reference intervals for endocrine markers, especially those as-

Table 5. Recommended laboratory best practices for transgender care.

Create a document that details your institution's protocol for identifying transgender patients. This document should specifically highlight the several systems that indicate sex assignments, examples of which include intake forms and IT[a] interfaces (LIS and HIS)
Do not cancel laboratory tests based on assumed sex specificity (e.g., hCG, PSA)
Ensure proper flagging of tumor and pregnancy related markers are implemented without sex specificity
Provide diversity training to the phlebotomy team and ensure gender incongruence is a component
Recognize that reference intervals for transgender patients have not been established and therefore hormone status and clinical judgment must be used to assess abnormal laboratory values

[a] IT, information technology.

sociated with sexual development and reproduction, also have age-specific values associated with their interpretation *(52)*. These age-related changes should be integrated when evaluating results for transgender individuals.

When ordering laboratory testing for testosterone there is often confusion as to whether total or free concentration is the most appropriate. Testing is further complicated by having both mass spectrometry (LC-MS/MS) and immunoassay (IA) options. There is controversy over which test and method are optimal for many clinical scenarios *(58)*. From a laboratory perspective, total testosterone by either LC-MS/MS or IA is preferred for most cases because we have standard reference methods/materials available and standardization has drastically improved *(59)*. Total testosterone by IA is best used for screening adult men and can be appropriate for transmen. Similarly, the IA can be used to confirm testosterone suppression after prostate cancer therapy, and therefore can be appropriate for transwomen.

Total testosterone measured by LC-MS/MS is the gold standard and is considered the best test for women, children, and men on testosterone replacement *(59)*. This assay is likely most appropriate for transmen and transwomen. Free testosterone has less utility because there is no gold standard method, no reference material, and no standardization, which muddles result interpretation *(60)*. The methods are dependent on either sex hormone binding globulin concentration–based calculations or using more complicated equilibrium dialysis methods that are not widely available. The best use of free testosterone is for confirming hypogonadism, and therefore it may have utility in transwomen depending on the clinical context.

Deutsch et al. used IA to compare free and total testosterone concentrations in transmen and transwomen after 6 months of hormone therapy *(56)*. The transwomen were evaluated against the age-matched female reference interval and transmen against the age-matched male reference interval. If their testosterone concentration fell into the compared interval they were labeled as being within the "physiologic range." For transwomen, 67% and 93% of subjects fell within the age-matched reference interval for total and free testosterone, respectively. For transmen, 71% and 65% of subjects fell into the age-matched reference interval for total and free testosterone, respectively. These differences in free and total testosterone did not necessarily indicate the gender phenotype.

Reference intervals for hemostasis are not defined by sex, but since exogenous hormone supplementation is known to increase risk of venous thromboembolism, laboratory values are important *(23, 24, 27)*. Venous thromboembolism has different mechanisms in transmen compared to transwomen. Testosterone increases platelet aggregation and increases hematocrit (transmen); estrogen increases protein C deficiency (transwomen). However, while the risk of venous thromboembolism in this population is higher, the incidence is low *(61)*. Hemophilia screening before initiating hormone therapy does not appear to be indicated.

Sex-specific reference intervals are clinically indicated for select laboratory tests. Establishing reference intervals applicable to the transgender population for these tests is important. The diversity embedded within this demographic makes population selection key to properly defining these intervals. For example, it may be best to establish CBC reference intervals using samples collected from a spectrum of transmen, such as those administered testosterone transdermally and intramuscularly. The subsets can be evaluated individually, and if consistent, combined. This approach will allow the clinical community to establish the appropriate intervals to define a healthy and pathological state in this demographic.

Future Directions

Utilization and interpretation of laboratory testing is an ever-evolving area of medicine because of the vast diversity in patient demographics. The transgender population has more recently been recognized as medically underserved *(13)*. Providing optimal and accessible healthcare for this group of people is important for overall population health *(62)*. The role of the laboratory in

attaining improved care for transgender people includes increasing the physiological understanding of hormone therapy and, to the extent possible, dismantling the sex binary that plagues most electronic medical systems and certain laboratory tests (Table 5).

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