New data on reference ranges for transgender men

Charna Albert

February 2021—Cisgender male reference intervals can be used to interpret testosterone concentrations in transgender and nonbinary adults on masculinizing therapy, but reference intervals specific to the transmasculine population should be used to evaluate estradiol, say the authors of a recently published study (Greene DN, et al. *J Appl Lab Med.* 2021;6[1]:41–50).

Participants in the study were 82 healthy transgender adults who had been prescribed testosterone for at least one year. The purpose of the study was to derive reference intervals for common endocrine laboratory measurements in adults on masculinizing gender-affirming hormone therapy. In addition to estradiol and free and total testosterone, the researchers measured LH, FSH, SHBG, prolactin, progesterone, AMH, and DHEAS. For estradiol, they derived a reference interval (by LC/MS/MS) with an upper limit of 168 pg/mL.

Most of the endocrine markers the researchers analyzed are not measured routinely in the transgender population, says Dina N. Greene, PhD, DABCC, clinical associate professor, University of Washington Department of Laboratory Medicine. "The gonadotropins are measured in fertility workups, unexplained amenorrhea, or other types of abnormalities that warrant an endocrine workup, whereas being trans doesn't warrant a complete endocrine workup in and of itself, contrary to popular belief," Dr. Greene says. But for total testosterone and estradiol, which are routinely monitored, the study gives "a pretty distinct recommendation."

Dr. Greene

Testosterone and estradiol are almost always evaluated before and after gender-affirming therapy is initiated, even if threshold concentrations are derived empirically, the authors write. In patients on masculinizing therapy, estradiol is commonly evaluated until cessation of menstruation and may continue to be measured thereafter, Dr. Greene says, "either because of curiosity or continued spot bleeding or because there is continued attenuation related to gender or hormones."

Using the cisgender male reference interval for estradiol of less than 45 pg/mL, approximately 18 percent of the cohort would have been flagged abnormally high, the authors write. Thus, study results show that "if you use cisgender male reference intervals to interpret estrogen concentrations in transgender men, you are setting unrealistic expectations," Dr. Greene says. And estradiol intervals for cisgender women are based on the menstrual cycle and "fluctuate wildly depending on the phase, so it's hard to compare estradiol concentrations in a trans guy on T to [those of] a cisgender woman." For estradiol, she says, "trans men need their own reference intervals."

"There's a consistent group, maybe it's a quarter, that you never get down to the cisgender male range for estradiol," says study coauthor Matthew D. Krasowski, MD, PhD, vice chair, clinical pathology and laboratory services, and clinical professor of pathology, University of Iowa Hospitals and Clinics. Dr. Krasowski shared details from the study during a CAP20 virtual session and in an interview with CAP TODAY. "There have been attempts to use estrogen blockers, but the literature on that doesn't seem promising, and now you're adding another drug that may have side effects." Then, too, hormone therapy isn't necessarily covered by insurance. "So the more complicated you make the treatment regimen, they may not be able to afford it."

In transgender adults on masculinizing therapy, it is standard of care to measure testosterone every three months

for the first year of therapy and at least annually thereafter. Study results contradict the Endocrine Society clinical practice guideline for the treatment of gender-dysphoric persons, which says a goal of masculinizing therapy should be to titrate testosterone concentrations to a range of 400–700 ng/mL (Hembree WC, et al. *J Clin Endocrinol Metab.* 2017;102[11]:3869–3903). Dr. Greene and her collaborators contend there is only empirical evidence to support the recommendation. It was arrived at by experts in clinical care, she says, "who are not the experts in setting reference limits that are broadly used by populations. That's where the clinical lab comes in." Study results do align with guideline recommendations to use the age-matched cisgender male reference intervals for testosterone.

"In talking to providers who manage these patients," Dr. Krasowski says, "they're not usually targeting specific concentrations. They are looking for effects that are gender affirming. Some patients clearly reach that below this range," while others reach it at higher levels. "If this was your narrow range, it could be frustrating."

In fact, Dr. Greene says, the suggested testosterone range "doesn't parallel the ranges we use for cisgender men," which have a higher upper reference limit. "And oftentimes we don't set an upper reference limit for cisgender men; it's just 'greater than' whatever the lower limit is." Putting forth a suggested range is unrealistic because it's difficult for patients to time their blood draw appointments relative to their most recent dose of testosterone, she says. "If someone has given themselves their weekly or bimonthly dose of testosterone that morning, their concentrations are going to be different than three days later, than a week later."

In general, the reason testosterone is measured is to help transgender men build a relationship with their gender and new sense of self, she says. Only in limited situations would it be monitored because concentrations are too high or low. "When you're working with the trans community, there are psychosocial factors you're trying to attenuate as well as attenuating hormones. So we have this measurement to show these guys, 'Look, physiologically you're looking like a man.' Whatever your definition of that is."

Dr. Greene has built specific tests that are identified as "testosterone for people on masculinizing hormones" or "testosterone for people on feminizing hormones."

"That at least allows the proper reference intervals to be appended," though it places the burden on providers to order the correct test, she says. At the University of Iowa Hospitals, Dr. Krasowski says, if patients have self-identified in the medical record as having a gender identity that is different than their legal sex, "we're currently suppressing reference ranges that are male- or female-specific. And we have a comment that indicates 'this may be affected by therapy.'"

"Ideally," Dr. Greene says, "we would have algorithms that incorporate gender and hormone use in order to append a reference interval that fits a specific population, but far and wide we are bad at that in the laboratory. We have strict binary systems for many things."

"Our systems are rigid," she adds, "but our interpretations don't have to be."

Other findings from the study show that cisgender male reference intervals can be used to evaluate SHBG in transgender men, and intervals for cisgender women can be used to interpret prolactin. For FSH, LH, progesterone, AMH, and DHEAS, the authors derived reference intervals that differ from those of cisgender men and women.

Progesterone, LH, and FSH are not monitored routinely in transgender people, but they may be evaluated as part of a fertility workup or for specific endocrine conditions. According to the study, the intervals derived for these analytes most closely resemble the follicular phase in cisgender women, with a higher upper reference limit compared with cisgender men but a lower upper limit compared with the ovulatory phase of cisgender women. These analytes may not be interpreted correctly if cisgender male or female reference intervals are applied, but because they are likely to be evaluated case by case and by endocrine or reproductive specialists rather than general practitioners, the authors do not believe that laboratories will need to implement measures to address these ranges. "It would be useful just to include the information," Dr. Krasowski says.

“These labs don’t exist in a silo,” Dr. Greene says of the tests. “You’re not looking at a progesterone concentration without a clinical picture,” or a complete hormone analysis. An isolated, slightly elevated progesterone in a transgender male patient would not concern her, she says, if everything else looked normal and the patient was satisfied with the results of therapy. But if a transmasculine patient was attempting to conceive and had recently stopped taking testosterone and had a low progesterone, she says, “I would look at that carefully as a trend over time and at what all the hormones are doing together.”

AMH, a marker of ovarian reserve, also is not typically measured in transgender people but may be evaluated in transmasculine patients who wish to conceive. Participants had a slightly higher upper reference limit relative to cisgender women—“an unexpected result,” the authors write, given that one of the treatment goals of masculinizing therapy is amenorrhea.

“We scratched our heads at this one,” Dr. Krasowski says, speculating that results may have differed with a larger or more age-diverse cohort. (The median age was 27.) In addition, no participants were on treatment regimens that included modalities other than testosterone—notable, he says, because in a prior study that found significant decreases in AMH, participants were receiving an aromatase inhibitor and a GnRH agonist in addition to testosterone (Caanen MR, et al. *Fertil Steril.* 2015;103[5]:1340–1345).

Total testosterone and estradiol were measured using immunoassay and liquid chromatography coupled with tandem mass spectrometry. FSH, LH, SHBG, prolactin, progesterone, AMH, and DHEAS were measured using immunoassay. LH, AMH, and DHEAS were measured on the Roche Cobas immunoassay instrument, while the other analytes, including total testosterone and estradiol, were measured on the Roche and Beckman Coulter DxI immunoassay instruments. Free testosterone was calculated.

The authors measured many of the analytes on multiple instruments to follow “in a limited way what’s been done in other areas, like pediatrics, where the samples are harder to get so you analyze them on multiple instruments,” Dr. Krasowski says.

The study of transgender men was preceded by an analysis of the same endocrine markers in a cohort of 93 transgender individuals on stable feminizing hormone therapy. In that study, conducted by the same group, the distribution of results differed from those of cisgender men and women across all measurements, supporting the use of transfeminine-specific reference intervals for the sex hormones (Greene DN, et al. *J Appl Lab Med.* 2021;6[1]:15–26).

More than a third of participants in the feminizing cohort administered spironolactone in addition to estrogen, and a little more than 10 percent administered estrogen and progesterone. Spironolactone administration was associated with statistically significant differences in the distribution of results for AMH, FSH, LH, and progesterone. “Spironolactone is a messy drug. It affects multiple hormones and also has effects on blood pressure and other endpoints,” Dr. Krasowski says. “So it’s tricky, what it might be doing. But there’s a lot of variability in how it’s prescribed, and it does seem to be causing some differences.” The study wasn’t sufficiently large to answer with certainty how spironolactone might impact the tests, he says.

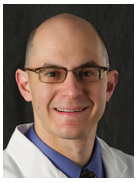
The researchers had hoped to recruit 120 participants to each of the two studies but ultimately fell short, Dr. Krasowski says. While it helped that the clinics in Seattle and Iowa City where recruitment took place had years of experience treating LGBTQ patients, it was a difficult recruitment, he says, noting that a longer recruitment period and travel reimbursements might have made it easier. One exclusion criterion—obesity—was the subject of debate, and a decision was made to stick with a BMI cutoff of less than 30. But it “cost us about 40 percent of potential subjects,” Dr. Krasowski says. Because of the limited sample size, they were unable to analyze subgroupings, such as age or mode of hormone administration. A limitation of the study of transgender men was that the timing of last testosterone dose was not reported, meaning participants may have been at different points in the cycle when measurements were taken.

Both studies are the first of their kind to prospectively derive transgender reference intervals, Dr. Krasowski says. “It’s kind of amazing that it’s taken this long.” Dr. Greene speculates it’s a lack of interdisciplinary communication

that resulted in a “less robust promotion of the need for this.” It’s also hard, she adds, to get oppressed populations to trust medical institutions.

The prospective studies made it possible to get a consistent range of tests that would have been difficult to pick up retrospectively, Dr. Krasowski says, as well as complete data on the subjects who were recruited. In a retrospective analysis of 150 transgender men and 152 transgender women that he helped author, there was a wide range in the completeness of lab monitoring, he says, driven in part by infrequent care or use of telehealth because of distance or insurance or other limitations (Humble RM, et al. *J Appl Lab Med.* 2019;3[5]:799–814).

Dr. Krasowski describes as complicated the existing research on laboratory changes in transgender patients, but says some things are clear. Most clear-cut are the findings related to hematological parameters, shown in multiple studies, he says. In a study he and Dr. Greene coauthored with others (Greene DN, et al. *Clin Chim Acta.* 2019;492:84–90), individuals prescribed testosterone or estrogen had hematology parameters that were not clinically different from those of cisgender males and females, respectively, regardless of serum hormone concentration. Thus, for individuals on gender-affirming hormone therapy, hemoglobin, hematocrit, and RBC count can be interpreted using the sex-specific reference intervals for their affirmed gender. “This is the clearest cut,” Dr. Krasowski says. “It moves to the opposite range.”



Dr. Krasowski

Dr. Krasowski highlighted findings from the 2019 retrospective study he and colleagues did and other retrospective studies. In adults on feminizing hormone therapy, increases in prolactin and SHBG have been observed. Creatinine and FSH decrease on balance, albeit with more variability seen across studies. The data on lipids, liver enzymes, DHEAS, FSH, LH, and AMH are inconsistent or limited, or both, or seen with complex effects.

In transgender men, creatine increases are consistent across studies, though that may be related mostly to increase in muscle mass, he says. Increases in ALT and AST also have been observed but not in all studies. DHEAS, FSH, LH, and SHBG tend to decrease, while the data on AMH are inconsistent or limited, or both, or seen with complex effects.

The effects of hormone therapy on plasma lipids is unclear. “Particularly for triglycerides, LDL, and total cholesterol, the changes are complex across studies,” Dr. Krasowski says, noting it may have to do with inclusion criteria, such as BMI. The data on HDL in studies of transgender men are fairly consistent, with multiple retrospective studies reporting decreases, but the data are less consistent in studies of transgender women.

Microbiology is likely to be a focus of future research, Dr. Krasowski says. “Are there changes in genitourinary flora due to hormones or surgery?” Dr. Greene and others have published the only clinical study on this topic to date, comparing the vaginal floras of transgender men on testosterone therapy with those of cisgender women. They found that the vaginal microbiome of transgender men may differ from that of cisgender women, with the transgender male participants in the study less likely to have *Lactobacillus* as their primary genus. They also had a significantly increased relative abundance of more than 30 species and a significantly higher alpha diversity according to the Shannon diversity index (McPherson GW, et al. *Clin Chem.* 2019;65[1]:199–207).

Further research is needed, too, to resolve how route of administration may affect laboratory values. Oral estradiol, for example, has a first pass through the liver, which doesn’t occur with intramuscular, subcutaneous, or topical administration. More research is also needed on the impact of additional medications such as spironolactone and flutamide.

Awaiting publication now, Dr. Greene says, are the results of a study on estrone, an estrogen metabolite sometimes used in transgender medicine, the clinical utility of which she says is highly controversial. “This article”—a study spearheaded by a pharmacist—“gives insight into the relative ratios of estrogen to estradiol, depending on the mode of administration of estradiol.”

In progress is a high-sensitivity troponin analysis by Drs. Greene and Krasowski and others. Studying the effects of gender-affirming hormone therapy on high-sensitivity troponin may shed light on the physiological mechanism that accounts for the sex-based difference in high-sensitivity troponin reference intervals.

“It’s unclear whether the sex-based differences in high-sensitivity troponin affect clinical outcomes,” Dr. Greene says, and how gender discrimination, like long-held assumptions that women present with atypical heart attack symptoms more often than men, “bleeds into evaluating people based on their feminine or masculine characteristics when they present with signs and symptoms of acute myocardial infarction.”

“There’s a lot that can be gleaned from looking at the distribution of high-sensitivity troponin in transgender populations, and how gender-affirming hormones do or do not shift high-sensitivity troponin concentration,” she continues. Says Dr. Krasowski: “There, having a reference range is important because you’re going to trigger off it. So we’ll see what that finds.”

Charna Albert is CAP TODAY associate contributing editor.