Transgender adult reference intervals taking shape

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November 2019—Current sex-specific intervals can be used to interpret hematology results for transgender people using their affirmed gender, say authors of a study published earlier this year.

In a session at the 2019 AACC annual meeting, Dina N. Greene, PhD, DABCC, technical director of Kaiser Permanente Washington laboratories and an author of the study, shared details of the analysis of 172 transgender people recruited between late 2017 and mid-2018 (Greene DN, et al. *Clin Chim Acta.* 2019;492:84–90). She also reported soon to be published sex hormone reference intervals for transgender adults on stable hormone therapy.

Dr. Greene said she was discouraged from starting the study by people who said the transgender population is too diverse, with the main concern being that some transgender people are on hormone therapy and others are not. Dr. Greene's response: "People are diverse. We define our population, get a set of reference intervals, and start somewhere. Then we see what else we have to do."

Dr. Greene and her colleagues collaborated with the University of Iowa Department of Pathology and recruited study participants from two LGBTQ-specific clinics in Seattle and Iowa City. Some of the data were published in *Clinica Chimica Acta*; other data are in preparation.

Study participants were healthy transgender adults who were at least 18 years old and on stable, gender-affirming hormone therapy for at least one year. "We excluded people if they were diabetics, obese, a current cigarette smoker, had a history of blood clots or cardiovascular disease, if they were HIV positive, had sleep apnea, or had a current pregnancy," Dr. Greene said.

Researchers tested participants' whole blood and serum. Measured were complete blood counts, testosterone, estrogen, SHBG, LH, FSH, AMH, progesterone, prolactin, electrolytes, lipids, and HbA1c.

'If you are a transgender man on stable hormone therapy, your reference intervals should look like cisgender men....'
Dina Greene, PhD, DABCC

"We collected samples from 79 transgender men and 93 transgender women, " she said. The average age of study participants was 28.8 years for transgender men and 35.1 years for transgender women. Participants used various modes of hormone administration; injection was the most highly favored method for testosterone—"very common because it's cheaper," she said—while more than half of the transgender women chose oral administration for their

estrogen. More than half of the transgender women also took an antiandrogen—spironolactone, progesterone, or finasteride—with their estrogen.

The distribution of sex hormone results for people on masculinizing hormones did not closely match cisgender male reference intervals. “The cisgender male intervals say anything greater than 200 ng/dL is normal” for total testosterone levels, Dr. Greene said, while the cisgender male reference range for estradiol is less than 48 pg/mL. If the cisgender reference limit fell outside the 95 percent confidence interval derived from transgender cohorts, the study says, reference change values were used to evaluate if the difference was clinically significant.

“The distribution of results for people on masculinizing hormones for these hormones is different,” Dr. Greene said. The free testosterone measurements, for example, “wouldn’t represent the central 95th percentile; this would represent the central 60th percentile. Therefore, these reference intervals don’t apply.”

Dr. Greene and her collaborators used immunoassays and mass spectrometry to measure sex hormones and concluded that “immunoassay is good enough,” she said. “When do we need mass spectrometry?” is a common question, she added. “For most of the time when you’re measuring this in a general trans male population, the cheap immunoassay is good enough.” And it is not necessary to measure free testosterone. “Total testosterone is generally fine” and will provide the required information.

The authors developed the following sex hormone reference intervals for people on masculinizing hormones: estrogen, less than 100 pg/mL; total testosterone, 180 to 900 ng/dL; and free testosterone, 15 to 170 pg/mL.

For the feminizing cohort, “we have a very similar picture. If you compare their reference intervals to that of cisgender women, they don’t quite apply,” she said.

“With free and total testosterone, it’s similar. There are big discrepancies in the concentration of total testosterone, but this would still be lower than the lower reference limit, so these would kind of group into the same clinical interpretation of, okay, this testosterone is low for this population.”

The authors developed the following sex hormone reference intervals for people on feminizing hormones: estrogen, 30 to 500 pg/mL; total testosterone, less than 200 ng/dL; and free testosterone, less than 20 pg/mL.

They used two immunoassays and looked at the lower limit of both. “For the most part, you’re going to group everything clinically the same whether or not you use mass spec or immunoassay.”

The reference intervals derived for the people on feminizing hormones will be published sooner than those for the masculinizing hormones, Dr. Greene said, adding, “Stay tuned.”

The study of hematology parameters—hemoglobin, hematocrit, and red cell count—for the masculinizing and feminizing cohorts showed good correlation, for the most part, with cisgender male and female reference values, respectively.

The distribution of results for hemoglobin concentration in the masculinizing cohort “fits perfectly, meaning that you can use the cisgender male range,” she said. The commonly used hemoglobin reference interval for cisgender men is 13.0 to 18.0 mg/dL; in the masculinizing cohort, the calculated hemoglobin reference interval was 12.8 to 17.4 mg/dL. “That hormone concentration is what is really driving hemoglobin concentration. This was the same for hematocrit and basically the same for red cell count.”

“If you are a transgender man on stable hormone therapy, your reference intervals should look like cisgender men, which is quite interesting.”

For study participants on feminizing hormones, it “ended up being the exact same story,” Dr. Greene said. “This is also interesting because we know that cisgender women have lower hematological parameters than cisgender men. And we know that testosterone stimulates erythropoiesis, but we also know women menstruate, so it’s been a little vague what the actual physiological mechanism is of the hemoglobin and red cell indices differences.” This

showed us, she said, that it is hormone driven. “The cisgender female reference interval fits nicely with this data and is similar for hematocrit and for red cell indices”—a “positive finding,” she added, “because it’s just easy.”

Dr. Greene said she was unable to easily incorporate the reference interval information for transgender patients into the KPWA laboratories’ electronic medical record system, which would accept only a binary gender. She created a workaround that requires appending a comment to the hematocrit test section for every CBC ordered. The comment reads: “For transgender, nonbinary, and gender diverse patients, reference intervals for Hgb, Hct, and RBC can be found here www.KPWAinternallink.com.” (The link will not work outside of KPWA laboratories.)

She created a similar workaround with a comment for the prolactin concentration test. That reference interval has not yet been published.

The comment for race as it relates to estimated glomerular filtration rate provided a precedent for such workarounds. “We already have one example in the lab where we try to include minority populations within the results, even though we don’t have the ability to know someone’s race in our LIS,” she said.

The solution was more complicated for testosterone and estrogen testing. “I couldn’t just append a comment because things are a lot different. So we built four new tests.” The test names are “testosterone for people on masculinizing hormones,” “testosterone for people on feminizing hormones,” “estrogen for people on masculinizing hormones,” and “estrogen for people on feminizing hormones.”

“We didn’t say, ‘for transgender men and transgender women,’ because there are nonbinary people. This was more inclusive.” And the new tests are straightforward, she said. The physician can order testosterone testing for people on masculinizing therapy and see the specific reference levels appended.

“This is helpful if you’re a transgender male, because most of the time you’re not going to suppress your estrogen down low enough that you’re going to look like a cisgender guy,” she said. The new test options present a more realistic picture to patients by showing how well their levels match those of other people who are trying to masculinize. “If the levels are much higher, that’s when you can start to look at aromatization and some other drugs that can be used to try to suppress estrogen.”

Dr. Greene presented the case of a 40-year-old transgender male blood transfusion patient who retained his uterus and ovaries and presented to an outside institution for heavy vaginal bleeding after cervical mass biopsies (Mays JA, et al. *Transfusion.* 2018;58[3]:823–825). The outside institution reported that the patient’s hemoglobin level was 6 g/dL.

“They transfused him but he kept bleeding,” she said. “They transferred him to the University of Washington and admitted him to the gynecology unit,” where the hemorrhage continued and a massive transfusion protocol was initiated.

Since the patient was listed in the EMR as male, the transfusion service laboratory immediately prepared a shipment of uncrossmatched type O, RhD-positive packed RBCs. This was problematic because “in general, when people are of childbearing ages and have a uterus and ovaries, we like to transfuse them with O-negative blood because they won’t have the alloreactivity if they have an Rh-positive fetus,” she said. A literature search on the subject of transgender transfusion produced “basically nothing.”

Sex considerations for transfusion medicine are fairly simple, she noted. “For recipients, it’s basically the type of blood, such as giving O-negative to people who may want to impregnate themselves. For the donor, it’s HLA alloantibodies; if you are an ICU patient, we prefer to transfuse blood of someone who has never been pregnant before because that can lead to adverse downstream effects.”

That sexual orientation is used as a risk prevention strategy in transfusion medicine creates donor barriers. “They don’t ask, ‘Have you been pregnant before?’ They look and say, ‘You’re female’ and then might ask if you’ve been pregnant. But if they perceive your gender or sex as male, they probably won’t ask. So you run the risk of giving the wrong blood to someone.”

In the case of the 40-year-old transgender male, the UW laboratory noticed that the order was placed from a gynecology floor for a male patient and called for clarification. The patient was transfused with the correct blood product: O, Rh-negative units. “Calling for clarification is not something we usually want,” she said. “If we had better EMR receptivity in the LIS, that could be avoided.”

Increased risk of thrombosis is an issue that comes up in patients on hormone therapy, but “it’s not as bad as one would think,” Dr. Greene said. Transgender women in particular are at increased risk of thrombosis, but the incidence is low. “The reason is estrogen causes procoagulant shifts,” she said. “Usually when you have very high concentrations of estrogen, you’re about to have a baby. There is a lot of blood so we want to be able to clot. One of the functions of estrogen is it has these procoagulant shifts that increase hemostatic factors and decrease antithrombotic factors.”

Study models with oral contraceptives or postmenopausal women on hormone replacement therapy are not equivalent to transgender women, she said.

In transgender men, testosterone stimulates erythrocytosis and erythropoiesis but has not been shown to increase the rates of venous thromboembolism.

“We wanted to answer the question of how bad the clotting risk is for transgender women and did a systematic review to assess VTE risk,” she said. A literature search of hundreds of studies left them with 12 that had data on outcomes of estrogen use in transgender women. “The end game was that, yes, transgender women do have a slightly increased risk of clotting, but it is basically about the same risk as someone who would be taking oral contraceptives.” Some variables, such as smoking or oral versus injected therapy, were not part of the 12 studies they reviewed. “But I like this study,” she said of the systematic review, “because it showed that one of the things we think to be the least safe is actually not as big of a deal.” Dr. Greene said more studies about clotting risk in transgender women are to come.

A recent survey of LIS companies provided insight into their ability to capture, retain, and display transgender patient information. Martha E. Lyon, PhD, DABCC, a clinical biochemist in the Department of Pathology and Laboratory Medicine, Royal University Hospital, Saskatchewan Health Authority, Saskatoon, speaking in the same AACC session, said she heard Dr. Greene speak a few years ago at a Canadian clinical chemistry meeting. “I realized I had a whole lot more I needed to learn about transgender patients and how we can provide a better service to them.”

She focused on laboratory information systems and their readiness to represent transgender patients. Dr. Lyon and her colleagues consulted a 2018 CAP TODAY article on LIS companies’ views of their customers’ needs and the accompanying LIS product guide.

“We took the contact information for the 30 companies and put together a very small survey,” Dr. Lyon said. Seven LIS companies responded quickly to the first survey email request she sent on April 22, and three more companies answered her follow-up request on May 22. All companies were informed that responses would be presented as pooled and anonymized data in the AACC annual meeting session.

“The first question we asked was, ‘Does your LIS/EMR product collect, retain, and display patient biological sex?’” That information can cue Pap tests and prostate exams, Dr. Lyon said, and it can be used for linking the laboratory test results or reference ranges. Seven companies said they collect patient biological sex information; one company said it did not.

(Two companies that didn’t respond to specific questions and instead gave a general response, Dr. Lyon said, are listed as not answering questions in the survey results.)

“We wanted to ask a little bit more about the information collected with respect to biological sex,” Dr. Lyon said, “and so we said, ‘If you collect [patient biological sex], is it male/female, male/female or other/unknown, or is it flexible? Can it be designed and customized for your clients?’”

‘We asked, ‘Are your customers requesting the capability of capturing more fields for transgender patients?’’
Martha Lyon, PhD, DABCC

“We found that the companies that responded were able to answer more than one of these,” she said. One company only had the option of a male/female category, six companies had male/female or other/unknown categories, and four companies also had an option for a flexible/customized-by-client category. “It was a combination for several of the companies.”

The next question asked whether companies collected, retained, and displayed patient gender information separately from patient sex information. Seven LIS companies said yes; one company said no.

“We went further and asked, ‘If you have the data field for assigned sex at birth, do you actually capture that information?’ Seven LIS companies said yes, and one company said no.”

For gender identity, three companies said they were capable of capturing this information, and five said they were not. “It will depend on the information being captured,” Dr. Lyon said. “Is it male to female? Is it gender choosing not to disclose? We never went into that level of depth with respect to the questionnaire.”

Dr. Lyon also asked about the ability to capture preferred patient name and pronouns. “We know that the gender identity field in the electronic health record is very important for clinical care, but we also know the importance, especially for frontline staff, to be able to appropriately address the patients. When we avoid misgendering the patients, it’s better care and more confidence-building for all individuals involved.”

A follow-up question asked how long it would take companies to add the ability to capture information on assigned sex at birth and gender identity. Five companies said they thought they would be ready in one to two years, while three companies were already able to capture that information.

Five companies said they planned to offer reference range fields for transgender patients. Three said they did not.

“We then asked, ‘Are your customers requesting the capability of capturing more fields for transgender patients?’” Dr. Lyon said. Two companies said yes and six said no, and that result surprised Dr. Lyon. “What this indicates is the need to promote more awareness of why it is important to be able to capture this information and to provide better service to our transgender patients. It’s very important within the laboratory medicine area.”

Several companies submitted comments about the future development or barriers in LIS/EMR systems as they relate to transgender patient representation. They found a major theme and a predictable one, Dr. Lyon said: reference ranges.

One company wrote that barriers include “the lack of industry standards and even the lack of industry consensus

as to how transgendered patient reference ranges should be managed . . . . There are not enough studies to create the patient populations to determine reference range standards."

Another company wrote, "For the laboratory to assume the responsibility for the interpretation of reference ranges based on birth sex with or without hormone therapy and with or without surgical procedures for a transgendered person may not be appropriate."

One company commented on the need for more research "to determine the appropriate reference ranges for transgender and gender-nonconforming populations. The effects of hormone replacement therapy and other medical interventions on target reference ranges is not well understood and preliminary research has yet to provide clear results."

Amy Carpenter Aquino is CAP TODAY senior editor.