Lab analysis in diabetes—a preview of what's to come

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November 2021—The guidelines for laboratory analysis in the diagnosis and management of diabetes mellitus are being revised and will be released next year. In a virtual session at the AACC meeting in September, laboratorians got a look at some of the recommendations to come.

"Long before there was a COVID pandemic there was a diabetes pandemic," said David B. Sacks, MB ChB, chair of the expert committee leading the revision and senior investigator and chief of the clinical chemistry service, NIH Clinical Center. He and a co-presenter—professor Åke Lernmark of Lund University—presented preliminary recommendations related to glycolysis, glycated protein, gestational diabetes mellitus, lab analysis in the diagnosis of type 1 diabetes onset, and genetic risk markers.

"We are at the stage where the recommendations are being graded, so we're fairly close to the completion of the initial draft of the document," said Dr. Sacks, who noted they intend to publish the guidelines in both *Clinical Chemistry and Diabetes Care*. The last set of such guidelines was published a decade ago (Sacks DB, et al. *Clin Chem.* 2011;57[6]:e1–e47; Sacks DB, et al. *Diabetes Care.* 2011;34[6]:e61–99).

The aim of the first recommendation Dr. Sacks highlighted is to minimize glycolysis, and it says a tube containing a rapidly effective glycolytic inhibitor, such as granulated citrate buffer, should be used. If this cannot be achieved, it says, the sample tube should immediately be placed in an ice-water slurry and subjected to centrifugation to remove the cells within 15 to 30 minutes. Tubes with only enolase inhibitors, such as sodium fluoride, should not be relied on to prevent glycolysis.

A new study "demonstrates very nicely the glycolysis in different tubes," Dr. Sacks said (Fischer MM, et al. *Clin Chem.* 2021;67[7]:1032–1034). In comparing the effects of glycolysis inhibitors, Fischer, et al., evaluated blood collected in six tubes: Three tubes contained either lithium heparin, EDTA, or sodium fluoride, and three other tubes contained citrate and were from different manufacturers. (Citrate-containing tubes, Dr. Sacks noted, are not available in the U.S. but are used in other countries, particularly in Europe.) The samples stood at room temperature and were centrifuged at various time points up to 24 hours.

The difference to the lithium heparinized tube at times zero was taken as the standard, and the findings revealed a statistically significant decrease in the lithium heparin and EDTA tubes at 30 minutes, Dr. Sacks said, with the decreases continuing uninterrupted. The sodium fluoride tube showed a statistically significant decrease at 15 minutes, but the rate of decline leveled off.

Among the three citrate tubes, the decreases became statistically significant at 15 minutes, four hours, or 24 hours, depending on the manufacturer, he said. "Even though it was statistically significant, the decrease was very, very small and unlikely to be clinically significant."

Another new recommendation says all pregnant women with risk factors for diabetes should be tested for undiagnosed prediabetes and diabetes at the first prenatal visit using standard diagnostic criteria.

"Gestational diabetes mellitus has been defined for many years as glucose intolerance first recognized during pregnancy," he said. "This has changed more recently."

Dr. Sacks

The rationale for the recommendation, Dr. Sacks said, is it's estimated globally about five percent of women ages 18 to 44 have diabetes, and about 50 percent of these women have not been diagnosed. About 24 percent of women of the same ages have prediabetes, Dr. Sacks said, and 90 percent of those women are undiagnosed. "If you combine those numbers, close to one third of women of reproductive age have impaired glucose metabolism, and the overwhelming majority are not aware they have this," he said.

The following standard recommendation continues: All pregnant women not previously known to have diabetes should be evaluated for GDM at 24 to 28 weeks of gestation.

A related new recommendation says women with GDM should be tested for diabetes four to 12 weeks postpartum using nonpregnant OGTT criteria exclusively. And another says lifelong screening for diabetes should be performed in women with a history of GDM using standard nonpregnant criteria at least every three years.

"The rationale is that women with GDM are at increased risk for type 2 diabetes, and recent estimates range from seven- to 12-fold," Dr. Sacks said. "And importantly, the cumulative evidence of type 2 diabetes after GDM is 50 to 60 percent, so it's important to monitor these people."

In the upcoming revision will be two new recommendations for glycated protein. One says in clinical settings where interfering factors compromise interpretation of HbA1c results, assays of other glycated proteins, such as fructosamine or glycated albumin, may be used. Another says HbA1c values that are inconsistent with the clinical presentation should be investigated further, and that a comparison of suspicious HbA1c results with other glycated protein assays may be informative.

"The rationale," Dr. Sacks said, "is that any factor that significantly alters red blood cell lifespan will alter hemoglobin A1c. So if the red cell lifespan is shortened, there will be less time for glucose to attach to hemoglobin to form glycated hemoglobin or hemoglobin A1c, so the hemoglobin and A1c will be lower than expected from the patient's average glucose."

Fructosamine and glycated albumin reflect glycation of serum proteins, he said, "and because of this, the value is independent of red blood cells."

"It should be noted that the half-life of albumin in the blood is considerably shorter than that of red blood cells, so they represent the average glycemia over 14 to 21 days rather than the couple of months represented by hemoglobin A1c."

Far less evidence is available for these glycated serum proteins than there is for hemoglobin A1c, Dr. Sacks said.

The time trends in incidence rate of type 1 diabetes in children worldwide is increasing in most locations, except Japan and Mexico, said expert committee member Dr. Lernmark of Lund and Skane University Hospital in Sweden. "In all other countries listed, there is a steady increase of the disease." The past decade has also seen significant advances in the research of biomarkers to predict type 1 diabetes in children.

"Only about 10 percent of newly diagnosed type 1 diabetes children and adults have a first-degree relative with the disease," he said. The propensity to acquire type 1 diabetes depends largely on genetic risk factors, and the human leukocyte antigen on chromosome 6 is the dominant risk factor, Dr. Lernmark said. The HLA DR-DQ genotype groups are the most important risk factors. At highest risk are children who are DR3-DQ2/DR4-DQ8.

Fifteen to 20 percent of newborn children have the propensity, if exposed to the trigger, to develop type 1 diabetes, he said.

DR4-DQ8 children tend to develop insulin autoantibodies as the first appearing autoantibody, and HLA DR3-DQ2 children tend to develop GADA autoantibodies as the first appearing autoantibody. To get the autoantibody, environmental factors are needed. "But this is not enough. Additional factors are needed, for example, genetic

factors outside the HLA system."

Sixty percent of children who develop a first autoantibody develop a second one within one year. According to the staging of autoimmune type 1 diabetes, stage one is two or more islet autoantibodies with normoglycemia. Stage two is two or more islet autoantibodies with dysglycemia. Stage three is the diagnosis of diabetes; the classical symptoms are associated only with this stage.

"We know from studies primarily in first-degree relatives that with one autoantibody, the risk for diabetes was about 10 percent over 10 years of follow-up," increasing to about 50 to 60 percent if there were two or more autoantibodies and even higher for three autoantibodies. "And from 2001 to 2013, we learned more, that no antibodies is no diabetes. If there is one islet autoantibody in the now general population among children who were born with the HLA risk, not first-degree relatives necessarily, then about 15 percent develop type 1 diabetes within 10 years. And two or more autoantibodies will result in almost 100 percent diabetes if you follow them for 20 years."

In the screening studies that are ongoing in many countries, where children are followed either from birth or there is screening of schoolchildren, diabetes in stage three is often diagnosed by OGTT, and the diagnosis is without the classical diabetes symptoms. "So many of the families participating in these clinical studies or screening studies are not experiencing ketoacidosis or dramatic onset of type 1 diabetes as it occurs in the general population," Dr. Lernmark said.

Type 1 diabetes is thought to have two endotypes: children who develop insulin autoantibodies as the first appearing autoantibody at age one to four, primarily in HLA DR4-DQ8 children, which may be related to enterovirus B prolonged shedding, and children who develop GADA autoantibodies at age two to three as the first autoantibody, primarily in DR3-DQ2 children. "The finding is that GADA as the first autoantibody is associated with a mastadenovirus infection, also reflecting prolonged shedding," Dr. Lernmark said.

In The Environmental Determinants of Diabetes in the Young (TEDDY) study, 4,543 genetically at-risk children were followed from birth to age 15 in Germany, Sweden, Finland, and the United States. Children were enrolled in the study as newborns between 2004 and 2010 and tested every three to six months for the development of islet autoantibodies and type 1 diabetes (Krischer JP, et al. *Diabetologia.* 2015;58[5]:980–987). "It appeared that the children were unable to clear the enterovirus and were shedding virus for as long as six to 12 months," after which the insulin autoantibodies appeared, Dr. Lernmark said.

In the second endotype, the pattern of the incidence of the first autoantibody is different from IAA. For the latter, it's a pronounced peak at ages one to four and decreasing over time in the TEDDY study. The GADA antibody acquires a plateau at age two to three and then remains stable.

In TEDDY, where children are followed for 15 years, "we have children who have throughout their early life given blood samples 38 to 40 times, being antibody negative," he said. "And then when they turn 13 or 14, GADA autoantibodies suddenly appear."

Once these autoantibodies are confirmed and stable, the number accelerates and the person progresses to stage three. "In persistently autoantibody-positive children, higher IAA and IA-2A levels, but not GADA levels, increase the risk for stage three type 1 diabetes," he said.

Islet autoantibody levels may drop to below the detection limit prior to the clinical onset of diabetes. "It is as if the immune system is recognizing or monitoring how many beta cells are left," Dr. Lernmark said. "And when there are no beta cells left, there is no point to continue to make these autoantibodies—very similar to neutralizing virus."

Dr. Lernmark

Risk scores are being developed to predict the time to stage three among subjects with multiple islet autoantibodies, he said. A study by the JDRF-IBM T1D consortium followed more than 10,000 children from seroconversion to clinical onset of type 1 diabetes (Anand V, et al. *Diabetes Care.* 2021;44[10]:2269–2276). Researchers followed the trajectories of disease progression based on the first autoantibody to appear: multiple, IAA, or GADA. In children with multiple autoantibodies first, progression from seroconversion to diabetes moves quickly over 15 years. In children with IAA first, seroconversion is earlier and progression to diabetes more rapid. In children with GADA first, progression is slower. "There is data now at the time of onset indicating that you may, by looking at the antibody pattern at the time of onset, predict backwards that this particular patient may have had IAA as the first autoantibody some 10 years ago, or GADA autoantibodies," Dr. Lernmark said.

HLA remains the major genetic risk factor for type 1 diabetes, and based on the association between HLA and single nucleotide polymorphisms, there are now more than 70 genetic factors associated with type 1 diabetes, Dr. Lernmark said.

The group at the University of Exeter Medical School "developed the first genetic score where you are weighting the different genetic factors into a genetic score, taking HLA and 41 different SNPs together," he said. Results from the TEDDY study show it is possible to select a genetic risk score based on a child's age and cumulative risk for one or more autoantibodies (Bonifacio E, et al. *PLoS Med.* 2018;15[4]:e1002548). "You can predict how many years it will take for those children to develop not diabetes but a first autoantibody," Dr. Lernmark said.

Important for the expert committee were the many monogenic diseases in childhood diabetes detected often at an early age or even at the time of clinical onset, he said. "The support to find these mutations in families where MODY [maturity-onset diabetes of the young] has been diagnosed is well developed throughout the world."

Dr. Lernmark presented the two sets of recommendations related to genetic markers and risk scores. The first says routine determination of genetic markers such as HLA genes or single nucleotide polymorphisms is not of value at this time for the diagnosis or management of patients with type 1 diabetes. Typing for genetic markers and the use of genetic risk scores is recommended for patients who cannot be clearly classified as having type 1 or type 2 diabetes. And for selected diabetes syndromes, including neonatal diabetes and MODY, valuable information including treatment options can be obtained with definition of diabetes-associated mutations.

The second set of recommendations is as follows:

- Islet autoantibodies are not recommended for routine diagnosis of diabetes.
- Standardized islet autoantibody tests are recommended in prospective studies of children at increased genetic risk for type 1 diabetes following HLA typing at birth or in first-degree relatives of type 1 diabetes patients.
- Routine screening for islet autoantibodies in patients with type 2 diabetes is not recommended at present.
- It is important that islet autoantibodies be measured only in an accredited laboratory with an established quality control program and participation

in a proficiency testing program.

“The future development is that there is interest now not only from researchers but from industry to qualify islet autoantibodies by FDA and the European counterpart, EMA,” Dr. Lernmark said. Once the islet autoantibodies are qualified as a certified biomarker, he said, industry will be able to use them in clinical trials to screen and to develop treatment approaches to prevent type 1 diabetes. And that, he said, is when islet autoantibody measurements will need laboratories’ close attention.

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