COMMENTARY

Alternate Ways to Quantify Antibodies

Kimber M. Simmons, MD, and Aaron W. Michels, MD

TYPE 1 DIABETES (T1D) IS NOW a predictable disease with the measurement of antibodies in the peripheral blood directed against proteins within insulin-producing β -cells.¹ There are currently four main biochemical islet autoantibodies measured that are directed against insulin, glutamic acid decarboxylase, islet antigen 2, and zinc transporter 8. The presence of two or more islet autoantibodies in children with a first-degree relative having T1D indicates pre-clinical T1D as nearly 100% of these children develop diabetes, marked by abnormal glucose homeostasis, provided long-term follow-up.²

Despite T1D being predictable, measuring biochemical islet autoantibodies remains a challenging task for several reasons. First, the method of determination greatly affects sensitivity and specificity.³ The gold standard determination for islet autoantibodies, a fluid-phase radioimmunoassay (RIA), has higher sensitivity and specificity than enzyme-linked immunosorbent assays; however, RIA requires the use of radioactivity to measure antibodies, which is not readily used in most diagnostic laboratories.^{4,5} Second, insulin autoantibodies are challenging to measure even in highly specialized laboratories using RIA for measurement. The sensitivity of measuring insulin autoantibodies can range from 22% to 57%, depending on the assay format used for measurement.⁶ Finally, venipuncture is generally required to obtain serum for antibody measurement, which can be difficult in young children.

In this issue of *Diabetes Technology & Therapeutics*, Bingley et al.⁷ begin to address the barrier of collecting blood for islet autoantibody measurements using dried blood spots (DBS) on filter paper to measure islet autoantibodies. Using the National Institutes of Health-sponsored TrialNet Pathway to Prevention Study,⁸ 229 children and young adult relatives of patients with T1D were recruited to obtain both DBS and serum for islet autoantibody determination using fluid-phase RIA. A capillary puncture (finger stick) was used to obtain whole blood as DBS collected on filter paper. The filter paper was then mailed to a central laboratory, where protein was eluted from the DBS samples and measured for islet autoantibodies. At the same visit, venipuncture was used to obtain serum, and the same thresholds for autoantibody positivity were used for both serum and DBS samples.

There was very good concordance between serum levels and those from DBS for glutamic acid decarboxylase, islet

antigen 2, and zinc transporter 8 antibodies; insulin autoantibodies were not measured using DBS on filter paper in this study. Overall, DBS measurements identified 42 of 44 (95%) relatives positive for multiple serum antibodies and correctly identified 145 of 147 (99%) autoantibody-negative subjects. These results were obtained despite only 55% of DBS samples being optimal, which was defined as having three out of five 1-cm-diameter circles completely filled with blood on filter paper. It is interesting that sample quality was highest in the youngest subjects (younger than 8 years of age) compared with adults. The authors concluded that the potential exists to use DBS measurements as a first-line screening test to identify individuals for T1D prevention trials.

One limitation of the current study was not assessing insulin autoantibodies from DBS on filter paper. As previously mentioned, measuring insulin autoantibodies is challenging, and the authors of the current study⁸ elected to not measure this autoantibody.⁹ However, insulin autoantibodies are generally the first antibody to appear in young children, and higher levels correlate with a shorter time to clinical T1D onset in multiple cohorts of children.^{10,11} Furthermore, oral insulin has shown promise in both primary and secondary T1D prevention trials¹²; the secondary prevention trials conducted through TrialNet include insulin autoantibody positivity as an enrollment criterion.¹³ For future screening efforts it is critically important to assess insulin autoantibody levels.

Determining the risk for T1D development in first-degree relatives has been done in several prospective longitudinal studies through serial measurement of islet autoantibodies. First-degree relatives identified through these screenings have a reduced rate of diabetic ketoacidosis (DKA) at diagnosis.¹⁴ Given that the incidence of T1D is increasing, that 85% of children who develop T1D have no family history, and that a large proportion of these children present to medical attention with life-threatening DKA, is it time to screen the general population?

T1D meets almost all of the conditions created by the World Health Organization for screening the general population for a disease: (1) the disease is an important health problem, (2) a treatment is available, (3) the natural history of the disease is understood, and there is a latency period (islet autoimmunity), (4) there is a test for the condition (measurement of islet autoantibodies) that is acceptable to the population, and (5) the cost of finding a case is economically

balanced in relation to medical expenditure.¹⁵ T1D easily satisfies the first three conditions, and the work presented in this issue of *Diabetes Technology & Therapeutics* begins to address the condition for an acceptable population-based screening test.⁷ The use of DBS on filter paper removes several obstacles to general population screening, such as the requirement for venipuncture, the shipment of serum or blood samples, and the need for multiple laboratories to perform technically challenging assays. As was done in the presented study, DBS on filter paper can easily be mailed to a central diagnostic laboratory for elution and measurement of islet autoantibodies using fluid-phase RIA. Additionally, measuring islet autoantibodies from DBS on filter paper has the potential to be used in several clinical and research environments, including pediatric practices, rural areas, and developing countries.

As islet autoantibody positivity can occur at various ages, yearly screening is important, and the cost of screening needs to be balanced against the medical costs of diabetes management and potential complications. With improved methods of islet autoantibody detection using electrochemiluminescence^{16–18} and “scaling up” the number of individuals screened, assay costs are expected to decrease. Furthermore, if the combination of screening and early diagnosis results in decreased DKA admissions or fewer complications such as severe hypoglycemia, this should reduce the overall T1D expenditure.

Several efforts are planned or underway to screen large pediatric populations for T1D risk. In the Denver metropolitan area, 200 children 2–6 years of age were screened at pediatric clinical practices for all four islet autoantibodies and antibodies directed against tissue transglutaminase (celiac disease).¹⁹ Serum was obtained by either a finger stick or venipuncture, with a finger stick the preferred method of sample collection. In Bavaria, the Fr1da study is underway to screen children at well-child visits for islet autoantibodies.²⁰ Integrating islet autoantibody assessments into established clinical practice has the potential to detect a high proportion of children who will develop T1D, to better understand disease pathogenesis, and to allow for T1D prevention trials.

It is our view that with the addition of insulin autoantibody measurements and an improved collection method, DBS on filter paper obtained by a simple capillary finger stick can be used as a screening test to assess the general population for T1D risk. As about 85% of all new-onset T1D patients do not have a family history of disease and DKA still remains a significant comorbidity, we are hopeful that large-scale screening efforts will lessen the burden of disease and eventually lead to T1D prevention.

Acknowledgments

This work was supported by grant DK095995 from the National Institute of Diabetes and Digestive Kidney Diseases.

Author Disclosure Statement

No competing financial interests exist.

References

- Atkinson MA, Eisenbarth GS, Michels AW: Type 1 diabetes. *Lancet* 2014;383:69–82.
- Ziegler AG, Rewers M, Simell O, et al.: Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. *JAMA* 2013;309:2473–2479.
- Bingley PJ, Williams AJ, Colman PG, et al.: Measurement of islet cell antibodies in the Type 1 Diabetes Genetics Consortium: efforts to harmonize procedures among the laboratories. *Clin Trials* 2010;7(1 Suppl):S56–S64.
- Torn C, Mueller PW, Schlosser M, et al.: Diabetes Antibody Standardization Program: evaluation of assays for autoantibodies to glutamic acid decarboxylase and islet antigen-2. *Diabetologia* 2008;51:846–852.
- Lampasona V, Schlosser M, Mueller PW, et al.: Diabetes Antibody Standardization Program: first proficiency evaluation of assays for autoantibodies to zinc transporter 8. *Clin Chem* 2011;57:1693–1702.
- Schlosser M, Mueller PW, Torn C, et al.: Diabetes Antibody Standardization Program: evaluation of assays for insulin autoantibodies. *Diabetologia* 2010;53:2611–2620.
- Bingley PJ, Rafkin LE, Matheson D, et al.: Use of dried capillary blood sampling for islet autoantibody screening in relatives: a feasibility study. *Diabetes Technol Ther* 2015; 17:867–871.
- Mahon JL, Sosenko JM, Rafkin-Mervis L, et al.: The TrialNet natural history study of the development of type 1 diabetes: objectives, design, and initial results. *Pediatr Diabetes* 2009;10:97–104.
- Bazzigaluppi E, Bonfanti R, Bingley PJ, et al.: Capillary whole blood measurement of islet autoantibodies. *Diabetes Care* 1999;22:275–279.
- Steck AK, Johnson K, Barriga KJ, et al.: Age of islet autoantibody appearance and mean levels of insulin, but not GAD or IA-2 autoantibodies, predict age of diagnosis of type 1 diabetes: Diabetes Autoimmunity Study in the Young. *Diabetes Care* 2011;34:1397–1399.
- Simell S, Hoppu S, Simell T, et al.: Age at development of type 1 diabetes and celiac disease-associated antibodies and clinical disease in genetically susceptible children observed from birth. *Diabetes Care* 2010;33:774–779.
- Bonifacio E, Ziegler AG, Klingensmith G, et al.: Effects of high-dose oral insulin on immune responses in children at high risk for type 1 diabetes: the Pre-POINT randomized clinical trial. *JAMA* 2015;313:1541–1549.
- Skyler JS, Krischer JP, Wolfsdorf J, et al.: Effects of oral insulin in relatives of patients with type 1 diabetes: the Diabetes Prevention Trial—Type 1. *Diabetes Care* 2005; 28:1068–1076.
- Barker JM, Goehrig SH, Barriga K, et al.: Clinical characteristics of children diagnosed with type 1 diabetes through intensive screening and follow-up. *Diabetes Care* 2004; 27:1399–1404.
- Simmons K, Michels A: Is it time to screen the general population for type 1 diabetes? *U.S. Endocrinology* 2015; 11:10–16.
- Yu L, Miao D, Scrimgeour L, et al.: Distinguishing persistent insulin autoantibodies with differential risk: nonradioactive bivalent proinsulin/insulin autoantibody assay. *Diabetes* 2012;61:179–186.
- Yu L, Dong F, Miao D, et al.: Proinsulin/insulin autoantibodies measured with electrochemiluminescent assay are the earliest indicator of prediabetic islet autoimmunity. *Diabetes Care* 2013;36:2266–2270.
- Miao D, Steck AK, Zhang L et al.: Electrochemiluminescence assays for insulin and glutamic acid decarboxylase auto-

antibodies improve prediction of type 1 diabetes risk. *Diabetes Technol Ther* 2015;17:119–127.

19. Gesualdo PD, Bautista KA, Waugh KC, et al.: Feasibility of screening for T1D and celiac disease in a pediatric clinic setting. *Pediatr Diabetes* 2015 August 6 [Epub ahead of print]. doi: 10.1111/pedi.12301.
20. Insel RA, Dunne JL, Ziegler AG: General population screening for type 1 diabetes: has its time come? *Curr Opin Endocrinol Diabetes Obes* 2015;22:270–276.

Address correspondence to:

Aaron W. Michels, MD

Barbara Davis Center for Childhood Diabetes

University of Colorado

1775 Aurora Court, A140

Aurora, CO 80045

E-mail: aaron.michels@ucdenver.edu