Published in final edited form as:

Diabet Med. 2017 July ; 34(7): 934–937. doi:10.1111/dme.13338.

Use of self-collected capillary blood samples for islet autoantibody screening in relatives: a feasibility and acceptability study

Y. Liu¹, L. E. Rafkin², D. Matheson², C. Henderson³, D. Boulware³, R. E. J. Besser⁴, C. Ferrara⁵, L. Yu⁶, A. K. Steck⁶, P. J. Bingley⁷, and the Type 1 Diabetes TrialNet Study Group

¹Division of Diabetes and Nutritional Sciences, King's College London, London, UK

²University of Miami Miller School of Medicine, Miami, FL, USA

³University of South Florida Health Informatics Institute, Tampa, FL, USA

⁴John Radcliffe Hospital, Oxford, UK

⁵Division of Pediatric Endocrinology and Diabetes, University of California, San Francisco, CA, USA

⁶Barbara Davis Center for Childhood Diabetes, University of Colorado School of Medicine, Aurora, CO, USA

⁷School of Clinical Sciences, University of Bristol, Bristol, UK

Abstract

Aims—To evaluate the feasibility of using self-collected capillary blood samples for islet autoantibody testing to identify risk in relatives of people with Type 1 diabetes.

Methods—Participants were recruited via the observational TrialNet Pathway to Prevention study, which screens and monitors relatives of people with Type 1 diabetes for islet autoantibodies. Relatives were sent kits for capillary blood collection, with written instructions, an online instructional video link and a questionnaire. Sera from capillary blood samples were tested for autoantibodies to glutamic acid decarboxylase, islet antigen-2, insulin autoantibody and zinc transporter 8. ‘Successful’ collection was defined as obtaining sufficient volume and quality to provide definitive autoantibody results, including confirmation of positive results by repeat assay.

Results—In 240 relatives who returned samples, the median (range) age was 15.5 (1–49) years and 51% were male. Of these samples, 98% were sufficient for glutamic acid decarboxylase, islet antigen-2 and zinc transporter 8 autoantibody testing and 84% for insulin autoantibody testing and complete autoantibody screen. The upper 90% confidence bound for unsuccessful collection was

Correspondence to: Polly J. Bingley. polly.bingley@bristol.ac.uk.
DR YUK-FUN LIU (Orcid ID : 0000-0002-8518-8756)

Supporting information

Additional Supporting Information may be found in the online version of this article: Appendix S1.

Competing interests

None declared.

A complete list of the Type 1 Diabetes TrialNet Study Group is included in Appendix S1.

4.4% for glutamic acid decarboxylase, islet antigen-2 and/or zinc transporter 8 autoantibody assays, and 19.3% for insulin autoantibody. Despite 43% of 220 questionnaire respondents finding capillary blood collection uncomfortable or painful, 82% preferred home self-collection of capillary blood samples compared with outpatient venepuncture (90% of those aged <8 years, 83% of those aged 9–18 years and 73% of those aged >18 years). The perceived difficulty of collecting capillary blood samples did not affect success rate.

Conclusions—Self-collected capillary blood sampling offers a feasible alternative to venous sampling, with the potential to facilitate autoantibody screening for Type 1 diabetes risk.

Introduction

Islet autoantibodies are indicators of β -cell autoimmunity and individuals with two or more islet autoantibodies have a >80% risk of developing Type 1 diabetes within 15 years [1]. A recent Type 1 diabetes staging classification system emphasized the importance of identifying islet autoimmunity in early pre-symptomatic stages, potentially allowing earlier intervention [2]. Prevention of progression to symptomatic disease requires large-scale screening to identify individuals at risk. Barriers to screening include geographical, cost and time constraints, as well as aversion to venepuncture, particularly in children. A simple test allowing self-collection of samples at home could overcome these limitations. Capillary sampling, having previously been validated against venous sampling for detection of islet autoantibodies [3–5], offers a potential solution. We assessed the feasibility and acceptability of home self-collection of capillary samples for autoantibody screening among relatives of people with Type 1 diabetes.

Methods

The TrialNet Pathway to Prevention study [6] recruited relatives from 15 US centres between August and December 2015. Institutional review boards at each centre provided ethical approval. Families provided written informed consent. Some centres offered telephone consultations, with postal return of consent forms. Samples were tested for glutamic acid decarboxylase (GAD), islet antigen-2 (IA-2), insulin and zinc transporter 8 (ZnT8) autoantibodies. GAD, IA-2, insulin autoantibody (IAA) and ZnT8 autoantibodies, in accordance with the Pathway to Prevention study protocol, replacing venous sampling. Participants positive for 1 autoantibody or with unsuccessful capillary sampling were recalled for confirmatory venous testing. Age-banded recruitment ensured enrolment of adequate numbers of younger children.

Capillary sampling kits, containing BD Microtainer[®] contact-activated lancets (Becton Dickinson, Franklin Lakes, NJ, USA), Sarstedt Microvette[®] serum gel capillary tubes with clotting activator (Sarstedt Inc., Newton, NC, USA) with written and online instructions, were provided in person or by post. A minimum volume of 200 μ l (up to 500 μ l) was requested. Adults performed the procedure on children aged <12 years, whilst children aged 12–17 years collected samples themselves, or were aided by an adult. Acceptability of collection was assessed by questionnaire (Appendix S1). Samples were returned to clinical sites by overnight courier. Extracted serum was stored and sent to the central laboratory at –20°C.

A radioimmunoassay was used to measure GAD, IA-2, ZnT8 and IAA in capillary serum samples in the TrialNet Core laboratory at the Barbara Davis Center for Childhood Diabetes, as previously described [6]. The same autoantibody thresholds were used for capillary serum as those used for venous serum in the TrialNet studies.

The primary outcome of the study was successful sample collection, defined as sufficient volume and quality (e.g. without excessive haemolysis) to allow definitive autoantibody results, including confirmation of positive results by repeat assay. Secondary outcomes incorporated acceptability of sample collection and additional analyses to formulate an upper confidence bound of unsuccessful sampling.

Results are presented as median (range) unless otherwise stated. The upper confidence bound of unsuccessful sampling was calculated using the Clopper–Pearson interval. Chi-squared tests were performed for categorical values. Non-parametric questionnaire scoring data were compared using Wilcoxon rank-sum and Kruskal–Wallis testing. Logistic regression was used to test for age effects in the risk of unsuccessful sample collection. *P* values <0.05 were considered significant. Statistical analysis was performed using SAS (SAS Inc.) and S-PLUS (TIBCO Software Inc.) software.

Results

Table 1 shows the demographic characteristics of the study participants. The median (range) interval between initial shipment and sample collection was 8 (0–104) days (*n*=158) and from sample collection to receipt at clinical centre it was 2 (0–7) days (*n*=169).

Rates of successful sample collection varied by autoantibody type (Table 1), with the highest success for GAD, IA-2 and ZnT8 autoantibody screening. There was no significant haemolysis in any samples.

The upper 90% confidence bounds of unsuccessful capillary sample collection were 4.4% for GAD, IA-2 and ZnT8 autoantibody assays combined, and 19.3% for IAA. Sampling was unsuccessful for 1 autoantibody assay in 16.0% of participants aged 8 years, 17.5% of those aged 9–18 years and 14.5% of those aged >18 years. There was no difference in the rate of unsuccessful sampling among age groups or overall age effect (*P*=0.73).

Of five participants with positive autoantibodies, four had confirmatory venous testing. Three showed fully concordant venous and capillary results and one was concordant for GAD and IA-2 autoantibody positivity, but an IAA positive capillary sample was negative on venous sampling. Of 39 individuals with unsuccessful capillary sampling, six have provided venous samples to date. All capillary samples were insufficient for IAA only and in subsequent venous samples one individual was IAA positive and five were IAA negative.

Capillary collection was considered uncomfortable or painful by 43% of participants. Nonetheless, 82% preferred home capillary sampling over outpatient venepuncture. Preference for capillary sampling varied by age: 90% of those aged 8 years, 83% of those aged 9–18 years and 73% of those aged >18 years, with greater preference among younger children (*P*=0.01). The median (interquartile range) score for ease of testing, using a scale

from 1 (easy) to 7 (difficult), was 3 (2–5), with no differences either among age groups [3 (2–5) in those aged < 8 years; 3 (1–4) in those aged 9–18 years; and 2 (2–5) in those aged >18 years ($P=0.39$)] or between respondents with successful compared with unsuccessful sample collection ($P=0.10$).

Written instructions were reported as easily understandable by 83.2% of participants. Only 53.6% watched the instructional video, with the rate of successful sampling being 76.9% in those who watched, compared with 85.9% in those who did not ($P=0.09$). There was no difference in reported difficulty of the procedure between those who watched the video, [median (interquartile range) 3 (2–4)] and those who did not [median (interquartile range) score 2 (2–5); $P=0.10$].

Discussion

Ease of sample collection has allowed the widespread adoption of capillary sampling in commercial self-testing for conditions such as hypercholesterolaemia and coeliac disease. Collection by clinicians has been used successfully for large-scale islet autoantibody testing in children [9], including those as young as 2 years [10,11]; however, no published data exist on self-collection of capillary samples for islet autoantibody testing outside the clinical setting.

Capillary samples have shown high concordance with venous samples when compared for GAD [3–5], IA-2 [3–5] (or combined GAD/IA-2 assays) [3,4], ZnT8 [5] and IAA[4]. Using a panel of GAD, IA-2 and ZnT8 autoantibody assays, we have previously shown that 95.5% of individuals who were multiple autoantibody-positive in venous serum were concordant in capillary samples collected as dried blood spots and 98.6% of those who were autoantibody-negative were concordant in dried blood spot samples [5].

Options for self-collection of capillary samples are direct collection of whole blood into tubes or dried blood spots on filter paper, with extraction of serum or eluates respectively. Both techniques offer sufficient stability to allow samples to be shipped at ambient temperature. Antibody levels are lower in dried blood eluates than in venous serum, particularly IAA, and weakly positive autoantibodies may be missed [3], introducing a risk of overlooking individuals with a single positive autoantibody. Our previous study, which did not include IAA in the initial screen, showed 39% of relatives who were single autoantibody-positive in a venous sample were missed by dried blood spot sampling [5]. Assay optimization on dried blood spots has improved detection of IAA[12], but collection of dried capillary samples, even by clinicians, resulted in variable quality, with 45% of samples insufficient to allow confirmation of positive results [5]. This limits adoption of dried blood spots in screening strategies, but collecting capillary whole blood into tubes could overcome these issues.

Of self-collected capillary samples, 16% were suboptimal for testing for all four autoantibodies because of insufficient volumes for IAA measurement, whereas only 3% were insufficient to measure GAD, IA-2 and ZnT8 autoantibodies. The confidence bounds for unsuccessful sample collection for GAD, IA-2 and ZnT8 autoantibody testing imply a

90% certainty that <4% would need retesting. Incorporating IAA potentially increases the rate of unsuccessful capillary sampling to 20%, largely because the more complex IAA radioimmunoassay requires fivefold greater volume than the other assays. Relatives were largely successful in collecting sufficient sample volumes to allow GAD, IA-2 and ZnT8 autoantibody testing. Previously, we showed that this panel identified multiple autoantibody-positive individuals with high sensitivity [5], the potential risk would therefore be missing individuals who are positive for IAA alone. A fail-safe capillary screening strategy would incorporate recall for venous sampling to confirm positive results and for otherwise autoantibody-negative individuals with insufficient sample for IAA testing. Requesting a higher minimum volume could more consistently provide sufficient volumes for all assays. Seroconversion for IAA tends to occur at an early age [13], which with current assays, may necessitate continued venous sampling to screen very young children.

Strong preferences were demonstrated for home capillary sampling over venepuncture at a clinical centre, particularly among families of children aged <8 years. Extending procedures for obtaining informed consent remotely by telephone or online could further facilitate testing. Our sampling kits appear user-friendly, and clear written instructions were adequate for the majority of testers, with few referring to video instructions. No data were collected from families who declined capillary sampling or unreturned samples, potentially leading to bias. Importantly, these families were already familiar with capillary glucose testing; therefore, the feasibility of capillary sampling in the general population requires further study.

Self-collected capillary blood samples are feasible and acceptable for autoantibody screening in individuals at risk of Type 1 diabetes, showing advantages over dried blood collection and preferred over outpatient venepuncture, particularly in children. With additional benefits of improving convenience and efficiency, home autoantibody testing through capillary sampling could aid Type 1 diabetes screening initiatives.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank all the families who took part in this study and the staff of the TrialNet Clinical Centres, who recruited participants and processed samples.

Funding sources

The sponsor of the trial was the Type 1 Diabetes TrialNet Study Group. The Type 1 Diabetes TrialNet Study Group is a clinical trials network funded by the National Institutes of Health (NIH) through the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Allergy and Infectious Diseases, and the Eunice Kennedy Shriver National Institute of Child Health and Human Development, through the cooperative agreements U01 DK061010, U01 DK061034, U01 DK061042, U01 DK061058, U01 DK085465, U01 DK085453, U01 DK085461, U01 DK085463, U01 DK085466, U01 DK085499, U01 DK085504, U01 DK085505, U01 DK085509, U01 DK103180, U01-DK103153, U01-DK085476, U01-DK103266 and the Juvenile Diabetes Research Foundation International (JDRI). The contents of this article are solely the responsibility of the authors and do not necessarily represent the official views of the NIH or the JDRI.

References

1. Ziegler A, Rewers M, Simell O, Simell T, Lempainen J, Steck A, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. *JAMA*. 2013; 309:2473–2479. [PubMed: 23780460]
2. Insel A, Dunne J, Atkinson M, Chiang J, Dabelea D, Gottlieb P, et al. Staging Presymptomatic Type 1 Diabetes: A Scientific Statement of JDRF, the Endocrine Society, and the American Diabetes Association. *Diabetes Care*. 2015; 38:1964–1974. [PubMed: 26404926]
3. Bazzigaluppi E, Bonfanti R, Bingley P, Bosi E, Bonifacio E. Capillary Whole Blood Measurement of Islet Autoantibodies. *Diabetes Care*. 1999; 22:275–279. [PubMed: 10333945]
4. Siraj E, Rogers D, Gupta M, Reddy S. A simple screening method for individuals at risk of developing type 1 diabetes: Measurement of islet cell autoantibodies (GADA, IA-2A, and IAA) on dried capillary blood spots collected on filter paper. *Horm Metab Res*. 2012; 44:855–860. [PubMed: 22893260]
5. Bingley P, Rafkin L, Matheson D, Steck A, Yu L, Henderson C, et al. Use of Dried Capillary Blood Sampling for Islet Autoantibody Screening in Relatives: A Feasibility Study. *Diabetes Technol Ther*. 2015; 17:867–871. [PubMed: 26375197]
6. Mahon J, Sosenko J, Rafkin-Mervis L, Lachin J, Thompson C, Bingley P, 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. [PubMed: 18823409]
7. Yu L, Robles D, Abiru N, Kaur P, Rewers M, Kelemen K, et al. Early expression of antiinsulin autoantibodies of humans and the NOD mouse: evidence for early determination of subsequent diabetes. *Proc Natl Acad Sci USA*. 2000; 97:1701–1706. [PubMed: 10677521]
8. Yu L, Boulware D, Beam C, Hutton J, Wenzlau J, Greenbaum C, et al. Zinc transporter-8 autoantibodies improve prediction of type 1 diabetes in relatives positive for the standard biochemical autoantibodies. *Diabetes Care*. 2012; 35:1213–1218. [PubMed: 22446173]
9. Strebelow M, Schlosser M, Ziegler B, Rjasanowski I, Ziegler M. Karlsburg Type I diabetes risk study of a general population: frequencies and interactions of the four major Type I diabetes-associated autoantibodies studied in 9419 schoolchildren. *Diabetologia*. 1999; 42:661–670. [PubMed: 10382585]
10. Raab J, Haupt F, Scholz M, Matzke C, Warncke K, Lange K, et al. Capillary blood islet autoantibody screening for identifying pre-type 1 diabetes in the general population: design and initial results of the Fr1da study. *BMJ Open*. 2016; 6
11. Ziegler A, Haupt F, Scholz M, Weininger K, Wittich S, Löbner S, et al. 3 Screen ELISA for High-Throughput Detection of Beta Cell Autoantibodies in Capillary Blood. *Diabetes Technol Ther*. 2016; 18:687–693. [PubMed: 27552135]
12. Simmons K, Alkanani A, McDaniel K, Goynes C, Miao D, Zhao Z, et al. Islet Autoantibody Measurements from Dried Blood Spots on Filter Paper Strongly Correlate to Serum Levels. *PLoS One*. 2016; 11
13. Ziegler A, Bonifacio E. Age-related islet autoantibody incidence in offspring of patients with type 1 diabetes. *Diabetologia*. 2012; 55:1937–1943. [PubMed: 22289814]

What's new?

- This is the first study to evaluate the use of capillary blood samples, collected at home by families themselves, for islet autoantibody testing.
- Capillary sampling was feasible and acceptable in all age groups including young children, with high rates of success in testing for glutamic acid decarboxylase, islet antigen 2 and zinc transporter 8 autoantibodies.
- The study highlights that insulin autoantibody assays were least likely to be successful, therefore, to avoid missing very young children at risk, second-line venous sampling may be required in this group.
- Screening of islet autoantibodies using this method is popular with families of people with Type 1 diabetes, particularly children.

Table 1Demographics and rates of successful capillary blood sampling, overall and by age group ($n=240$)

	Overall	Age		
		8 years ($n=81$)	9–18 years ($n=97$)	>18 years ($n=62$)
Median (range) age, years	15.5 (1–49)			
Gender, n (%)	121 (51.5)	39 (49.4)	56 (57.7)	26 (44.1)
Male	114 (48.5)	40 (50.6)	41 (42.3)	33 (55.9)
Female	5	2	0	3
Missing				
Overall successful sample rate, n (%)	201 (83.8)	68 (84.0)	80 (82.5)	53 (85.5)
GAD/ZnT8/IA-2 autoantibodies successful sample rate, n (%)	234 (97.5)	80 (98.8)	93 (95.9)	61 (98.4)
Insulin autoantibody successful sample rate, n (%)	202 (84.2)	68 (84.0)	81 (83.5)	53 (85.5)

GAD, glutamic acid decarboxylase; ZnT8, zinc transporter 8; IA-2, islet antigen-2.

Successful capillary blood sampling was defined as obtaining samples of sufficient volume and quality (e.g. without excessive haemolysis) to allow definitive autoantibody results, including confirmation of positive results by repeat assay.