International Journal of Clinical Trials
Patel SK et al. Int J Clin Trials. 2022 Nov;9(4):293-299
http://www.ijclinicaltrials.com
pISSN 2349-3240 | eISSN 2349-3259

DOI: https://dx.doi.org/10.18203/2349-3259.ijct20222690

Protocol

Immunophenotype and genetic risk scores to improve autoantibody negative type 1 diabetes classification: study protocol

Shivani K. Patel[1,2]*, Cindy S. Ma[2,3], Kirstine J. Bell[4,5], Richard Oram[6], William A. Hagopian[7], Spiros Fourlanos[8], Jerry R. Greenfield[1,2]

[1]Department of Diabetes and Metabolism, [3]Human Immune Disorders, Garvan Institute of Medical Research, Sydney, NSW, Australia
[2]St Vincent's Clinical School, Faculty of Medicine, UNSW Sydney, Sydney, NSW, Australia
[4]Charles Perkins Centre, Sydney Medical School, NSW, Australia
[5]The Children's Hospital at Westmead Clinical School, Faculty of Medicine and Health, University of Sydney, Australia
[6]Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter, United Kingdom
[7]Pacific Northwest Research Institute, Seattle, Washington, USA
[8]Department of Diabetes and Endocrinology, Royal Melbourne Hospital, Melbourne, VIC, Australia

Received: 05 June 2022
Revised: 19 July 2022
Accepted: 20 July 2022

***Correspondence:**
Dr. Shivani K. Patel,
E-mail: s.patel@garvan.org.au

ABSTRACT

Background: An estimated 10-30% of type 1 diabetes (T1D) individuals do not have detectable autoantibodies at diagnosis, thus are classified as "idiopathic" or "non-immune." Given the non-pathogenic role of islet autoantibodies, the validity of excluding an immune basis for disease in such individuals needs to be questioned. The pan-autoantibody negative type 1 diabetes in adults (PANDA) study aims to characterise the immune, clinical and metabolic phenotype of autoantibody negative T1D individuals.
Methods: This is a two-part, multi-centre study which is recruiting 100 participants: autoantibody positive T1D (N=25), autoantibody negative T1D (N=25), latent autoimmune diabetes in adults (N=25) and age- and sex-matched normoglycaemic control (N=25) individuals. Study 1 involves baseline pathology collection and high dimensional immune-phenotyping using flow cytometry. DNA will be extracted from saliva samples to calculate type 1 diabetes genetic risk scores (T1DGRS). Autoantibody negative individuals will undergo monogenic diabetes testing. Study 2 is a prospective, longitudinal sub-study of study 1 participants within 5 years of diagnosis. Beta cell function will be assessed using glucagon stimulated C-peptide at 0, 9 and 18 months. The primary outcome of study 1 is to determine the phenotype of immune cells in autoantibody positive and negative T1D compared to healthy controls. Secondary outcomes of study 1 include clinical and metabolic characteristics and the T1DGRS. The primary outcome of study 2 is the rate of decline of stimulated C-peptide over time.
Conclusions: The PANDA study is the first study of its kind which aims to improve diagnosis and characterisation of autoantibody negative T1D.

Keywords: Autoantibody-negative, Type 1 diabetes, Autoimmune diabetes, Immune phenotype, Characterisation

INTRODUCTION

Assessing Islet autoantibody status is useful in the prediction, diagnosis and subclassification of type 1 diabetes (T1D). While autoantibodies are detectable in majority of individuals with T1D, up to 20% do not have detectable serum autoantibodies at diagnosis.[1-4] This subgroup has been classified as 'idiopathic' as the aetiology of their insulin deficiency remains poorly understood.[5] Hence, this form of T1D is excluded from most clinical trials in the absence of biomarkers to confirm their diagnostic classification. It remains unclear whether autoimmune T-cell mediated beta-cell destruction contributes to insulin deficiency in autoantibody negative T1D. With several factors contributing to fluctuating autoantibody concentrations and specificities, an inability to detect serum autoantibodies does not exclude immune-mediated disease. Islet autoantibodies levels may fluctuate over time in some and others may harbour autoantibodies that are yet to be characterised. Alternatively, individuals with autoantibody-negative T1D may have a pre-disposed beta cell prone to immune or metabolic stress. We postulate that metabolic triggers such as weight gain may promote insulin resistance and precipitate immune-mediated beta cell destruction, in which case T2D therapies may be beneficial as adjunctive therapies. The role of insulin resistance in progression of T1D has been documented in autoantibody-positive first-degree relatives.[6,7] Accurate diagnosis from disease onset has important clinical implications for treatment in these individuals. In the absence of detectable autoantibodies, some individuals may be misclassified as type 2 diabetes (T2D) and commence non-insulin therapies, while others with an undiagnosed monogenic diabetes mutation may be subject to lifelong insulin.[8,9] Limited literature to date has found autoantibody negative T1D to be associated with older age at diagnosis, higher visceral adiposity, higher stimulated C-peptide and increased likelihood of T2D family history.[1,10-12] However, it remains difficult to distinguish whether some of these participants may in fact have T2D. Several studies have attempted to identify clinically meaningful immune-based biomarkers (largely T-cell based) to aid T1D prediction, diagnosis and prognosis. Efforts have been limited by few autoreactive T cells in the periphery, assays yet to be standardised and validated, and overlapping phenotypes with non-diabetes individuals.[13] To date, the comparison of the immune phenotype of autoantibody negative and autoantibody positive T1D remains to be studied. The T1D genetic risk score (T1DGRS) is a novel tool incorporating single nucleotide polymorphisms (SNP) in the Human Leukocyte Antigen (HLA) and non-HLA loci which have been associated with T1D.[14] It has been shown to be particularly useful in the prediction of T1D in addition to other factors such as number of autoantibodies, family history and BMI.[15] In particular, it has shown an ability to discriminate between diabetes subtypes including T1D, T2D and monogenic diabetes.[14,15] In 83% of individuals with discordant clinical features and autoantibody results, the T1DGRS correctly classified which individuals are likely to become insulin deficient.[15] Hence, the potential utility of the T1DGRS as an adjunct in the diagnosis and classification algorithm of autoantibody negative T1D is promising, and requires further research. Given the need to improve characterisation of autoantibody negative T1D, the PANDA study aims to elucidate the differences in the immune, metabolic and clinical phenotype of autoantibody negative compared to autoantibody positive T1D. We hypothesise that autoantibody negative T1D will have a similar immune phenotype to autoantibody positive T1D, however clinically a subset may have misclassified T2D, thus resulting in an intermediate phenotype. We also hypothesise that their rate of decline in beta-cell function, measured using a glucagon stimulated C-peptide, will be slower than autoantibody positive T1D. In those with misclassified T2D, stimulated C-peptide levels are likely to be markedly raised compared to T1D.

Aim and objectives

Aim and objective of study 1 was to determine whether the lymphocyte/immune cell phenotype differs in T1D individuals with or without autoantibodies, to compare baseline clinical characteristics (including age at diagnosis, sex, BMI, family history, HbA1c, body composition) between autoantibody positive and negative T1D individuals and to assess the utility of the T1DGRS in the diagnosis and subclassification of T1D. Aim and objective of study 2 was to compare the insulin secretory capacity and progression over time in T1D individuals with and without autoantibodies.

METHODS

Study design

This is two-part multi-centre study involving the Garvan Institute of Medical Research, St Vincent's Hospital Sydney and the Royal Melbourne hospital. Study 1 is a cross-sectional comparison of T1D with and without autoantibodies, latent autoimmune diabetes in adults (LADA) and age- and sex-matched normoglycaemic controls. LADA is differentiated from autoantibody positive T1D based on a clinical phenotype often resembling T2D, with detectable islet autoantibodies. This study will compare the immune phenotype across groups and assess the utility of the T1DGRS in differentiating between different subtypes. Study 2 is a prospective, longitudinal study comparing the insulin secretory capacity over time of recently diagnosed autoantibody positive and negative T1D individuals. Recruitment commenced for both studies in March 2021 with anticipated completion by September 2023.

Setting and study recruitment

Study 1: participants will be recruited from Sydney and Melbourne. T1D and LADA individuals will be alerted of

the study by their treating physician. If interested, they will be asked to contact the study co-ordinator via telephone or email. Participants will be sent a participant information sheet via email or post. Due to COVID-19 restrictions, they will be screened via a telephone survey to confirm eligibility (Table 1).

Table 1: Inclusion and exclusion criteria for study groups

Criteria
Group 1 (autoantibody negative T1D)
Adults 18-55 years of age with clinical diagnosis of T1D
Treated with insulin within 6 months of diagnosis
Symptomatic hyperglycaemia or DKA at presentation
Age of diagnosis <50 years of age
BMI <27 kg/m^2
Lack of GAD, IA2, ZnT8 and insulin antibody positivity at diagnosis OR within 7 years of diagnosis and autoantibody status at diagnosis unavailable
Group 2 (autoantibody positive T1D)
Adults 18-55 years of age with clinical diagnosis of T1D
Treated with insulin within 6 months of diagnosis
Symptomatic hyperglycaemia or DKA at presentation
Age of diagnosis < 50 years of age
BMI <27 kg/m^2
One or more positive autoantibody specificities (GAD, IA2, ZnT8, insulin) OR within 7 years of diagnosis and antibody status at diagnosis unavailable
Group 3 (LADA)
Adults 18 - 80 years of age with clinical diagnosis of LADA (autoantibody positive, non-insulin requiring for at least 6 months)
One or more positive autoantibody specificities (GAD, IA2, ZnT8, insulin)
Group 4 (non diabetic controls)
Age, sex and BMI matched to individuals with type 1 diabetes (groups 1 and 2)
Negative islet cell autoantibodies
Without evidence of diabetes
Exclusion criteria
Pregnant women
Individuals using immunosuppression and/or glucocorticoids
Active infection (clinical or elevated C-reactive protein)
Individuals with a history of haematological malignancy, bone marrow pathology or HIV

If eligible, they will be consented to participate in the study. A baseline survey relating to a participant's initial pathology at diagnosis will be sent electronically to the treating physician to complete. Healthy control will complete a baseline telephone survey with the study team. A total of 100 participants will be recruited (N=25 autoantibody negative T1D, N=25 autoantibody positive T1D, N=25 LADA and N=25 age-matched healthy controls). Remote pathology collection through a commercial pathology service will be arranged for each participant, negating the need for study visits. A saliva collection kit will be mailed to participants, with collection requested as per manufacturer instructions. Samples will be returned to the Garvan Institute Clinical Research Facility and stored at room temperature. DNA extraction will be performed at the Garvan Institute and sent to the Charles Perkins Centre to perform T1DGRS.

Study 2: Study 1 participants within 5 years of diagnosis will be invited to participate in study 2. Given the need for body composition assessment, post-menopausal women will be excluded from study 2. For participants residing in New South Wales (NSW), glucagon stimulation tests (GSTs) will be performed at the clinical research facility, Garvan Institute of Medical Research or through the Translational Research Centre, St Vincent's Hospital. Body composition scans will be performed at St Vincent's Clinic Bone Densitometry and Fibroscans at St Vincent's Public Hospital, Sydney. For participants residing in Victoria (VIC), glucagon stimulation tests were performed at Royal Melbourne Hospital. Body composition will not be assessed in these individuals. Glucagon stimulation tests will be repeated at 9 and 18 months from their initial study visit.

Eligibility

In individuals without documented autoantibody negativity at diagnosis, recruitment will be limited to those within 7 years of diagnosis to minimise the likelihood of autoantibody remission (Table 1). While the timing of remission and contributory factors remain unclear, this has been shown to occur particularly in individuals with longstanding diabetes duration.[16,17]

Screening and baseline surveys

Study 1: screening and baseline survey data will be collected remotely. Telephone surveys with participants will be arranged to assess eligibility, to consent eligible individuals and to co-ordinate remote pathology collection (Table 2). In eligible participants, further history relating to diabetes presentation, medication history, past medical history and family history will be recorded. Pathology collection for participants receiving vaccination or recovering from recent illness, will be deferred for a minimum of 3 weeks to ensure this does not confound their flow cytometry results. A secure, web-based portal link (REDCap) will be sent to the individual's treating endocrinologist or physician to obtain data relating to their participant's pathology including autoantibody testing, HbA1c at diagnosis and recent pathology.

Study 2: during the baseline study visit for study 2, participants will sign the study informed consent form. Height and weight will be measured. An intravenous GST will be performed. A body composition scan using DXA (Lunar Prodigy GE-Lunar) to calculate total body fat, free fat mass and central abdominal fat. Fibroscans will be performed to assess liver steatosis and stiffness will be performed following the GST (Table 2).

Table 2: Outline of study interventions and visits.

Interventions	Study 1 (all participants)	Study 2		
	Enrolment	Visit 1 (0 month)	Visit 2 (9 months)	Visit 3 (18 months)
Eligibility assessment	✓	✓		
Informed consent	✓	✓		
Blood sample	✓	✓	✓	✓
Saliva sample	✓			
Glucagon stimulation test		✓	✓	✓
Height		✓		
Weight		✓	✓	✓
DXA		✓ (NSW only)		
Fibroscan		✓ (NSW only)		

Baseline pathology

Blood samples will be tested for islet cell autoantibodies including glutamic acid decarboxylase-65 (GAD; Maglumi GAD65 chemiluminescence immunoassay), insulinoma-antigen 2 (IA2; Maglumi anti-IA2 chemiluminescence immunoassay), islet cell autoantibodies (ICA; Bio-Diagnostics Anti-ICA immunofluorescent antibody), zinc transporter 8 (ZnT8; Euroimmun Anti-Znt8 enzyme immunoassay), and insulin (IAA) autoantibodies (SydPath radioimmunoassay), C-peptide (Attelica IM), fasting glucose and HbA1c. Total IgA, IgG, IgM to screen for potential antibody deficiency and C-reactive protein will be measured to screen for concurrent infection or inflammation which may affect flow cytometry results.

High dimensional flow cytometry

Approximately 30 ml of blood will be sent to the biobanking services at the centre for Applied Medical Research in Darlinghurst, Sydney for processing and freezing down of peripheral blood mononuclear cells (PBMCs). Samples will be transferred to the Garvan Institute for analysis and storage. NK, T and B cell analysis will be performed by deep high-dimensional immunophenotyping using a 28-colour antibody panel developed for the BD FACSymphony.[18] This panel will provide an in-depth analysis of innate like lymphocytes (NK, gd T cells, MAIT cells) as well as naïve and memory T and B cell compartments, including $CD4^+$ T chelper susbsets such as circulating T follicular helper cells (cTfh), Th1, Th2, Th17 cells, and regulatory T cells (Tregs). Based on initial phenotyping results, further testing on B amd T cell function will be considered.

Type 1 diabetes genetic risk score

A saliva sample will be collected to extract DNA and calculate an individual's GRS (this includes SNP's from high-risk HLA genotypes and other common genetic variants associated with T1D). This will be performed in collaboration with the Charles Perkins Centre, Sydney.

Monogenic diabetes testing

All autoantibody negative participants will be invited to undergo testing for monogenic diabetes (Exeter Genomics Laboratory, United Kingdom), as autoantibody negativity has been identified as a discriminatory factor aiding monogenic diabetes diagnosis.[9]

Glucagon stimulation test

Beta-cell function in T1D participants will be assessed using intravenous glucagon stimulation C-peptide.[19,20]

Participants will need to fast for a minimum of 10 hours overnight. A slight reduction in long-acting insulin may be considered with an aim to achieve fasting blood glucose level between 4 and 12 mmol/l before the test. In participants with glucose level between 12 and 15 mmol/l, a correction dose of insulin may be administered a minimum of 2 hours before the test to achieve a target glucose of 10 mmol/l. In participants with a blood glucose level above or below 4 to 15 mmol/l, the test will need to be rescheduled. On arrival to the Garvan Institute Clinical Research Facility at 8 AM, a fingerprick glucose level will be checked prior to commencement of the test. A 21G intravenous cannula will be inserted in the antecubital vein. Glucose and C-peptide will be collected at -1 minute prior to intravenous injection of 1 mg glucagon (Glucagen, Novo Nordisk) (Table 3).

Table 3: Intravenous glucagon stimulation test procedure.

Time	Procedure
-1 min (baseline)	Glucose, C-peptide (2x samples)
0 mins	Inject 1mg IV glucagon
+6 mins	Glucose, C-peptide (2x samples)
+36 mins	Participant may be discharged if well

Repeat glucose and C-peptide will be collected 6 minutes following injection of glucagon. Participants will be observed for a further 30 minutes, to ensure nil adverse reactions are experienced. GSTs, HbA1c and islet autoantibody testing will be repeated at 9 and 18 months from baseline visit. Delta C-peptide (CP 6 min-CP fasting) divided by delta glucose (glucose 6 min-glucose fasting) will be used to as an index of beta-cell function.

Sample size calculation

Study 1: a minimum sample size of 100 participants (25 per group) will be recruited. Given no prior studies have specifically investigated T cell subset frequencies between autoantibody positive and negative T1D, this estimate was based on prior studies which have compared T cell subset frequencies between T1D and normoglycaemic controls.[21-23]

Study 2: The primary outcome of study 2 will be to assess the rate of decline in stimulated C-peptide over time. The target sample size for this study was based on power calculations using prior data from Aguilera et al (ref) in which glucagon stimulation tests were performed in Type 1A (N=8) and atypical type 1 diabetes (N=16) groups.[5] Based on these calculations, the proposed sample size of 20 participants with autoantibody negative and 20 autoantibody positive T1D will provide an 80% chance of detecting a difference of 0.24 nmol/l (270 pmol/l) allowing for a 20% drop out rate. A p value of <0.05 will be considered statistically significant.

Statistical plan

Clinical data will be collated on REDCap and extracted into SPSS Statistics for further analysis. Baseline characteristics will be summarised based on the data subtype. Normal distribution will be checked using skewness and kurtosis. Parametric data will be summarised using a mean with standard deviation and non-parametric data using median with an interquartile range. Differences between groups will be detected using analysis of variance for normally-distributed continuous variables, and using Kruskal Wallis tests for non-normally distributed continuous variables.

DISCUSSION

This is the first study to compare the immune cell phenotype of peripheral blood in autoantibody negative and positive individuals with T1D, in comparison to healthy age-, sex- and BMI-matched normoglycaemic controls. T-cell based biomarkers including increased Tfh cell frequency, increased CD183+Th1 cell frequency and increased Treg cell (CD4+CD25+) frequency have been previously identified in autoantibody positive T1D.[21,24] We hypothesise that autoantibody negative T1D will have a similar immune phenotype to autoantibody positive T1D, however, a subset of individuals with misdiagnosed T2D may result in an intermediate group phenotype. If an immune-based biomarker characterising this subtype is identified, it may facilitate accurate diabetes diagnosis, treatment and classification at disease onset. HLA genotypes associated with T1D risk have also been associated with T1D subtypes, although not routinely tested in clinical practice. According to the American Diabetes Association classification, high-risk HLA genotypes are thought to be absent in autoantibody negative T1D.[5] However, studies have identified such high-risk alleles in autoantibody negative individuals, resulting in reclassification to ‘autoimmune’ T1D despite negative autoantibodies.[8,11]

The T1DGRS is a novel tool which incorporates HLA and non-HLA SNP’s associated with T1D.[15] In some aspects, it supersedes the limitations of autoantibodies which may fluctuate or reduce over time.[17,25] The incorporation of the T1DGRS in the PANDA study is a useful adjunct which may help decipher whether there is an immune basis for disease in autoantibody negative T1D. With the advent of reduced genotyping costs, if clinically meaningful, this tool has potential to be routinely used in the diabetes diagnostic algorithm in individuals with atypical or discordant features of diabetes.[15] Few studies have compared stimulated C-peptide values between T1D subgroups, a surrogate marker for beta-cell function.[10,11] Based on these studies, autoantibody negative T1D was associated with higher C-peptide. However, C-peptide was not prospectively measured beyond 12 months of diagnosis. Furthermore, while differences in BMI were found, body composition was not thoroughly assessed.

This is of particular importance in autoantibody negative T1D, as prevalence is increased in non-Caucasian ethnicities, thus ethnicity-specific BMI cut-offs may need to be used.[4,26] The PANDA study will calculate total body fat, free fat mass and central abdominal fat using a DXA scan and assess liver stiffness and steatosis using a fibroscan. We hypothesise that autoantibody negative individuals with T1D will have greater total fat mass (and BMI), implying a potential greater role for insulin resistance in their diabetes aetiopathogenesis. It is plausible that beta cells in autoantibody-negative T1D may be pre-disposed to autoimmune destruction, with disease onset precipitated by metabolic stress or insulin resistance. Insulin resistance has already been identified as an independent risk factor for classic T1D.[6] Understanding the role of insulin resistance in this subgroup is of particular relevance given its implications in consideration of adjunctive therapies with an aim to reduce long-term cardiometabolic risk. This study has been strategically designed to facilitate progress despite the COVID-19 pandemic. For some patients, participation may lead to re-classification of their diabetes and thus alter their management. Whilst study findings will characterise an Australian population and may not be directly applicable to other populations, it has potential to improve our diagnostic framework and promote the need for adjunct, personalised, treatment options.

CONCLUSION

This study will aid understanding of this idiopathic subtype of diabetes, often assumed to have a non-immune basis. If there is supportive evidence for an immune basis for this form of diabetes, inclusion of autoantibody negative T1D in T1D immunomodulatory trials should be considered. In the event that their rate of beta-cell destruction is found to be slower, perhaps this subgroup may respond better to novel beta-cell preservation therapies. With better characterisation of clinical and metabolic markers and incorporation of adjunct tools, such as the T1DGRS or a robust immune cell biomarker, the diagnostic algorithm may be improved to better classify autoantibody negative T1D. Enhanced T1D subtyping may ultimately lead to more effective precision therapy.

ACKNOWLEDGEMENTS

Authors are thankful to university postgraduate award programme (University of New South Wales). Rebecca Hickey will be assisting with Fibroscans.

Funding: This project is funded by the Sr Mary Bernice, Packer Family Foundation Research Grant, through the St Vincent's Clinic Foundation
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Bravis V, Kaur A, Walkey HC, et al. Relationship between islet autoantibody status and the clinical characteristics of children and adults with incident type 1 diabetes in a UK cohort. BMJ Open 2018;8:e020904.
2. Classification of diabetes mellitus. Available at: https://www.who.int/publications/i/item/classification-of-diabetes-mellitus. Accessed on 20 October 2021.
3. Vipin VP, Zaidi G, Watson K, et al. High prevalence of idiopathic (islet antibody-negative) type 1 diabetes among Indian children and adolescents. Pediatr Diab. 2021;22:47-51.
4. Tiberti C, Buzzetti R, Anastasi E. Autoantibody negative new onset Type 1 diabetic patients lacking high risk HLA alleles in a Caucasian population: are these Type 1b diabetes cases? Diab Metab Res Rev. 2000;16:8-14.
5. Classification and diagnosis of diabetes: standards of medical care in diabetes-2021. Diab Care. 2021;44:S15-33.
6. Fourlanos S, Narendran P, Byrnes GB, Colman PG, Harrison LC. Insulin resistance is a risk factor for progression to Type 1 diabetes. Diabetol. 2004;47:1661-7.
7. Wilkin TJ. The accelerator hypothesis: a review of the evidence for insulin resistance as the basis for type I as well as type II diabetes. Int J Obesity. 2009;33:716-26.
8. Hameed S, Ellard S, Woodhead HJ. Persistently autoantibody negative (PAN) type 1 diabetes mellitus in children. Pediatr Diab. 2011;12:142-9.
9. Carlsson A, Shepherd M, Ellard S. Absence of Islet autoantibodies and modestly raised glucose values at diabetes diagnosis should lead to testing for MODY: lessons from a 5-year pediatric Swedish national cohort study. Diab Care. 2020;43:82-9.
10. 1Guarnotta V, Vigneri E, Pillitteri G, Ciresi A, Pizzolanti G, Giordano C. Higher cardiometabolic risk in idiopathic versus autoimmune type 1 diabetes: a retrospective analysis. Diab Metab Syndrome. 2018;10.
11. Aguilera E, Casamitjana R, Ercilla G, Oriola J, Gomis R, Conget I. Adult-onset atypical (type 1) diabetes: additional insights and differences with type 1a diabetes in a european mediterranean population. Diab Care. 2004;27:1108-14.
12. Catarino D, Silva D, Guiomar J. Non-immune-mediated versus immune-mediated type 1 diabetes: diagnosis and long-term differences-retrospective analysis. Diab Metab Syndrome. 2020;12.
13. Ahmed S, Cerosaletti K, James E. Standardizing T-Cell biomarkers in type 1 diabetes: challenges and recent advances. Diabetes. 2019;68:1366-79.
14. Patel KA, Oram RA, Flanagan SE. Type 1 Diabetes genetic risk score: a novel tool to discriminate monogenic and type 1 diabetes. Diabetes. 2016;65:2094-9.

15. Oram RA, Patel K, Hill A. A Type 1 Diabetes Genetic risk score can aid discrimination between type 1 and type 2 diabetes in young adults. Diab Care. 2016;39:337-44.
16. Richardson CC, Dromey JA, McLaughlin KA. High frequency of autoantibodies in patients with long duration type 1 diabetes. Diabetologia 2013;56:2538-40.
17. Williams GM, Long AE, Wilson IV. Beta cell function and ongoing autoimmunity in long-standing, childhood onset type 1 diabetes. Diabetologia. 2016;59:2722-6.
18. Payne K, Li W, Salomon R, Ma CS. OMIP-063: 28-Color Flow Cytometry Panel for Broad Human Immunophenotyping. Cytometry A. 2020;97:777-81.
19. Faber OK, Binder C. C-peptide Response to Glucagon: A Test for the Residual -cell Function in Diabetes Mellitus. Diabetes. 1977;26:605-10.
20. Fourlanos S. Latent autoimmune diabetes in adults : new clinical, immunogenetic and metabolic perspectives. J Univ Melbourne. 2006.
21. Kenefeck R, Wang CJ, Kapadi T. Follicular helper T cell signature in type 1 diabetes. J Clin Invest. 2015;125:292-303.
22. Xu X, Shi Y, Cai Y. Inhibition of Increased Circulating Tfh Cell by Anti-CD20 monoclonal antibody in patients with type 1 diabetes. PLoS One. 2013;8:e79858.
23. Oras A, Peet A, Giese T, Tillmann V, Uibo R. A study of 51 subtypes of peripheral blood immune cells in newly diagnosed young type 1 diabetes patients. Clin Exper Immunol. 2019;198:57-70.
24. Menart-Houtermans B, Rütter R, Nowotny B. Leukocyte profiles differ between type 1 and type 2 diabetes and are associated with metabolic phenotypes: results from the german diabetes study (GDS). Diab Care. 2014;37:2326-33.
25. So M, O'Rourke C, Bahnson H, Greenbaum C, Speake C. Autoantibody Reversion: changing risk categories in multiple-autoantibody–positive individuals. Diab Care. 2020;43:191731.
26. Speake C. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004;363:157-63.

Cite this article as: Patel SK, Ma CS, Bell KJ, Oram R, Hagopian WA, Fourlanos S, et al. Immunophenotype and genetic risk scores to improve autoantibody negative type 1 diabetes classification: study protocol. Int J Clin Trials 2022;9(4):293-9.