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ARTICLE

Prevention of diabetic ketoacidosis in relatives screened for islet autoantibodies and followed up in the TrialNet Pathway to Prevention study at a single institution in Italy

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Abstract

Aims/hypothesis Screening for islet autoantibodies is an effective method for identifying individuals with pre-symptomatic (stage 1 and 2) type 1 diabetes. This approach offers a valuable opportunity for education and monitoring, which can help to reduce the severity of clinical manifestations at clinical onset (stage 3), including diabetic ketoacidosis. The aim of the study was to evaluate the progression to stage 3 and the incidence of diabetic ketoacidosis in relatives of individuals with type 1 diabetes screened and followed up at a single institution in Italy.

Methods This was a single-centre observational study conducted at San Raffaele Hospital, Milan, Italy, within the international multisite TrialNet Natural History Study-Pathway to Prevention. First-degree (aged 1–45 years) and second-degree (aged 1–20 years) relatives were screened primarily for GADA, IAA and IA-2A. In the event of a positive result, subsequent testing was conducted for ICA and ZnT8A. Periodic autoantibody testing, metabolic monitoring and educational support were offered to all autoantibody-positive participants. Participants were screened between July 2005 and February 2020, with the latest update obtained between January 2023 and June 2024.

Results In total, 4046 relatives were screened at a median (IQR) age of 17.6 (7.9–38.0) years. At first screening, 4.9% were found to be positive, with 3.1% having a single autoantibody and 1.8% multiple autoantibodies. Follow-up data were available for 78.5% of the participants, with a median (IQR) follow-up time of 9.9 (6.5–13.5) years. Progression to stage 3 was observed in 51 (1.6%) participants. Disease onset occurred in 0.4% of autoantibody-negative, 6.5% of single-positive and 43.1% of multiple-positive participants after a median (IQR) time of 7.8 (5.4–10.4), 7.9 (2.1–11.8) and 2.9 (0.9–6.5) years, respectively (p=0.012). The Kaplan–Meier survival free of clinical diabetes at 15 years was 99.5% (95% CI 99.1, 99.7), 87.3% (95% CI 74.4, 94.0) and 45.9% (95% CI 31.1, 59.6), respectively (p<0.001). At the time of disease onset, no occurrences of diabetic ketoacidosis were documented. Median (IQR) HbA_{1c} was 64 (52–86) mmol/mol (8.0 [6.9–10.0]%) and median (IQR) venous pH at onset was 7.37 (7.35–7.39). Hospitalisation occurred in 22 paediatric participants, as part of standard practice for newly diagnosed patients at our institution aiming to provide disease education and insulin therapy optimisation.

Conclusions/interpretation The early identification of individuals at risk for type 1 diabetes through a single-centre approach, combining autoantibody screening and regular monitoring, completely prevented diabetes-associated ketoacidosis at disease onset in relatives of individuals with type 1 diabetes.

Trial registration ClinicalTrials.gov NCT00097292

Keywords Autoantibody · Diabetic ketoacidosis · Monitoring · Prevention · Screening · Type 1 diabetes

Abbreviation

DKA Diabetic ketoacidosis

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Research in context

What is already known about this subject?

- Diabetic ketoacidosis is an acute, potentially life-threatening complication associated with type 1 diabetes onset
- Diabetic ketoacidosis incidence is still relevant, with rates ranging from 21% to 44% in different countries worldwide (41% in Italy)
- Screening for type 1 diabetes-related autoantibodies can reduce the incidence of diabetic ketoacidosis at clinical onset

What is the key question?

- Can diabetic ketoacidosis be fully prevented?

What are the new findings?

- Diabetic ketoacidosis was completely prevented in a cohort of relatives of individuals with type 1 diabetes who underwent prior autoantibody screening
- This achievement highlights the role of a single-centre approach to the management and monitoring of at-risk individuals

How might this impact on clinical practice in the foreseeable future?

- This study demonstrates how linking people at risk of type 1 diabetes to a structured medical-care pathway can improve patient outcomes and guide more effective preventive strategies in clinical practice

Introduction

Type 1 diabetes is a chronic autoimmune disease, characterised by a prolonged pre-symptomatic phase of a gradual deterioration in pancreatic beta cell function, ultimately leading to insulin deficiency and hyperglycaemia [1]. Detectable markers of this clinically silent process are circulating islet autoantibodies, currently used in large screening programmes to identify individuals at risk of developing type 1 diabetes, stage the disease and predict its subsequent progression. Type 1 diabetes specific autoantibodies include the antigenically defined GADA, IAA, IA-2A, ZnT8A and ICA [2]. Islet autoantibodies usually develop sequentially. The presence of two or more of these markers in children or adolescents without diabetes is indicative of the pre-symptomatic phase of type 1 diabetes [3]. The disease is further classified through three evolving stages: stage 1 (normoglycaemia); stage 2 (dysglycaemia); and stage 3, corresponding to overt hyperglycaemia [4].

In multiple-autoantibody-positive children and adolescents, the risk of progression from stages 1 and 2 to stage 3 within 10 years is significant, reaching 80–90%, depending on age and genotype. Positivity for a single islet autoantibody, although not classified as a pre-symptomatic stage, is associated with a risk of progression to stage 3, approaching 15% at 10 years [3]. Conversely, prediction is much less clear in adults [5].

In the general population, the high prevalence of stage 3 type 1 diabetes new-onset cases in association with diabetic ketoacidosis (DKA) represents a serious public health concern, with rates ranging from 21% to 44% in different countries worldwide. In Italy, the prevalence of DKA at stage 3 type 1 diabetes diagnosis is 41.2% [6]. Conversely, among screened relatives of individuals with type 1 diabetes, the identification of pre-symptomatic type 1 diabetes together with education and metabolic monitoring has been shown to significantly reduce the incidence of DKA at disease onset, below 5% in most studies and down to 2.5% in some [7–13].

DKA is an acute, potentially life-threatening, complication that is frequently the consequence of a late diagnosis and a more advanced insulin deficiency. For most of the affected individuals, hospitalisation is needed, with some requiring intensive care. Furthermore, DKA has a negative prognostic effect on long-term metabolic control [14–17] and cognitive function [18, 19], so prevention of DKA at onset is a major goal in current type 1 diabetes management.

From July 2005 to February 2020, our institution was a member of the TrialNet Consortium, acting as an international centre in Italy. During this period, we participated in the Natural History [20] and the subsequent Pathway to Prevention [21] screening study for first- and second-degree relatives of people with type 1 diabetes.

The aim of the present study was to assess the incidence of stage 3 type 1 diabetes and associated DKA at onset

in a cohort of Italian relatives, reflecting a single-centre approach that combined screening, monitoring and follow-up in accordance with the study protocol recommendations.

Methods

All data reported in this cohort study refer to the participation of our institution, San Raffaele Hospital, Milan, Italy, as a TrialNet international centre in the Natural History Study [20], subsequently renamed Pathway to Prevention (ClinicalTials.gov registration no. NCT00097292) [21]. Participants were enrolled between July 2005 and February 2020, with the latest update performed between January 2023 and June 2024.

All individuals participating in the study, including parents or legal guardians, were required to provide informed consent. Additionally, minors were also required to assent to participate. Participation was voluntary and no specific selection with regard to sex/gender, ethnicity, regional and socioeconomic factors was considered. The study was approved by the local Ethics Committee and conducted in accordance with the principles set forth in the Declaration of Helsinki.

The recruitment and enrolment of participants in the study were directed towards relatives of individuals newly diagnosed with type 1 diabetes at the Paediatric and Adult Diabetes Units of San Raffaele Hospital. Additionally, screening was promoted both internally and externally by patient associations, research foundations and web-based communities of individuals with type 1 diabetes and their families.

Individuals with relatives diagnosed with type 1 diabetes were eligible for inclusion in the study, specifically those aged 1–45 years with a first-degree relative or aged 1–20 years with a second-degree relative affected by the disease. At the time of informed consent signature and blood drawing, anagraphic and anthropometric data, including age, sex and gender, were assessed by the healthcare professional. A preliminary blood sample was obtained for the measurement of GADA, IAA and IA-2A. In the event of a positive result for any of these autoantibodies, further measurement of ICA and ZnT8A (the latter starting in 2012) was performed. Children who tested autoantibody negative and were younger than 18 years of age were offered autoantibody re-screening annually. All confirmed autoantibody-positive individuals were provided with information regarding the potential symptoms of the disease and were invited to participate in periodic (every 6–12 months) monitoring for autoantibodies, HbA_{1c} and 2 h OGTT. All laboratory analyses were conducted at the TrialNet core laboratories. Families were encouraged to maintain a close contact with study personnel in the event of any symptom potentially related to the disease.

Furthermore, in order to update the follow-up, all participants or their parents/carers were either telephoned (at least two attempts by two designated physicians) or e-mailed between January 2023 and June 2024. Specifically, information was collected about the development of type 1 diabetes, clinical details of disease onset, the necessity of hospitalisation and occurrence of DKA. A review of the hospital files of study participants diagnosed with stage 3 type 1 diabetes at San Raffaele Hospital was conducted to investigate the cases in question. Individuals diagnosed at other medical facilities or by other physicians were invited to submit relevant documentation, including reports, notes or any pertinent material. Particular attention was placed on the collection of data pertaining to the occurrence of concomitant DKA. Type 1 diabetes and DKA were diagnosed according to ADA [22] and ISPAD [23] criteria.

Statistical analysis Continuous variables were summarised by medians (IQRs), as their distribution was confirmed to be non-normal, while categorical variables were expressed as absolute counts (n) and percentage. Between-group comparisons for continuous variables were performed using the Mann–Whitney U test (for comparison of two groups) or the Kruskal–Wallis test (for comparison of multiple groups), as appropriate. Categorical variables were compared using the χ^2 test. To account for multiple hypothesis and adjust statistical significance, Dunn's test was used for post hoc comparisons after a significant Kruskal–Wallis test, and Bonferroni correction was applied after a significant χ^2 test.

The incidence of progression to stage 3 type 1 diabetes was estimated and stratified according to the number of autoantibodies detected at the initial screening (0, 1 and ≥2 autoantibodies). Survival analysis was performed according to autoantibody stratification using the Kaplan–Meier estimator, and the results were compared by logrank test. Our primary aim was to assess the incidence of stage 3 type 1 diabetes onset and associated DKA. Other measures were also calculated, including the prevalence of autoantibodies stratified by three age groups (<10, 10–17 and ≥18 years) and the age at seroconversion. Missing values were not imputed. Statistical analyses were performed using Stata 18.0 (StataCorp, College Station, TX, USA). A p value <0.05 was considered as statistically significant.

Results

Prevalence and distribution of autoantibodies In total, 4046 participants were included in the analysis. Participants' characteristics at baseline are shown in Table 1. The median (IQR) age was 17.6 (7.9–38.0) years and 54.6% were female. The majority of participants (68.2%) were over 10 years of

Table 1 Baseline characteristics of the study population (*N*=4046)

Characteristic	Overall	No. of autoantibodies reported at first screening			*p* value
		0	1	≥2	
No. of participants	4046	3849	124	73	
Age, years, median (IQR)	17.6 (7.9–38.0)	17.6 (7.8–38.0)	21.2 (8.9–39.4)	13.8 (8.1–27.3)	0.15
Age group, *n* (%)					0.03
<10 years	1286 (31.8)	1227 (31.9)	34 (27.4)*	25 (34.2)*	
10–17 years	762 (18.8)	721 (18.7)	19 (15.3)	22 (30.1)	
≥18 years	1998 (49.4)	1901 (49.4)	71 (57.3)	26 (35.6)	
Female sex, *n* (%)	2211 (54.6)	2116 (55.0)	58 (46.8)	37 (50.7)	0.15
GADA-positive, *n* (%)	156 (3.9)	0 (0.0)	87 (70.2)	68 (93.2)	<0.001
IA-2A-positive, *n* (%)	39 (1.0)	0 (0.0)	7 (5.6)	32 (43.8)	<0.001
IAA-positive, *n* (%)[a]	58 (1.4)	0 (0.0)	26 (21.0)	32 (43.8)	0.001
ICA-positive, *n* (%)[b]	52	0	2 (1.6)[c]	50 (68.5)	
ZnT8A-positive, *n* (%)[d]	32	0	2 (1.6)[c]	30 (41.1)	

[a]Data missing for one individual due to an uninterpretable laboratory result

[b]Data missing for 3849 individuals as ICA test was performed only in autoantibody-positive participants according to protocol

[c]ICA and ZnT8A analysed as IAA result was uninterpretable

[d]Data missing for 3894 individuals as ZnT8A test was performed only in autoantibody-positive participants according to protocol

The *p* values related to between-three-groups comparison are derived from the Kruskal–Wallis test for continuous variables and the χ^2 test for categorical variables; **p*<0.05 between groups, identified through post hoc pairwise comparisons; for antibody-type comparisons, the reported *p* value corresponds to the pairwise comparison between single-positive and multiple-positive groups; *p* values for the between-group comparison of ICA vs ZNT8 are not presented due to low sample size

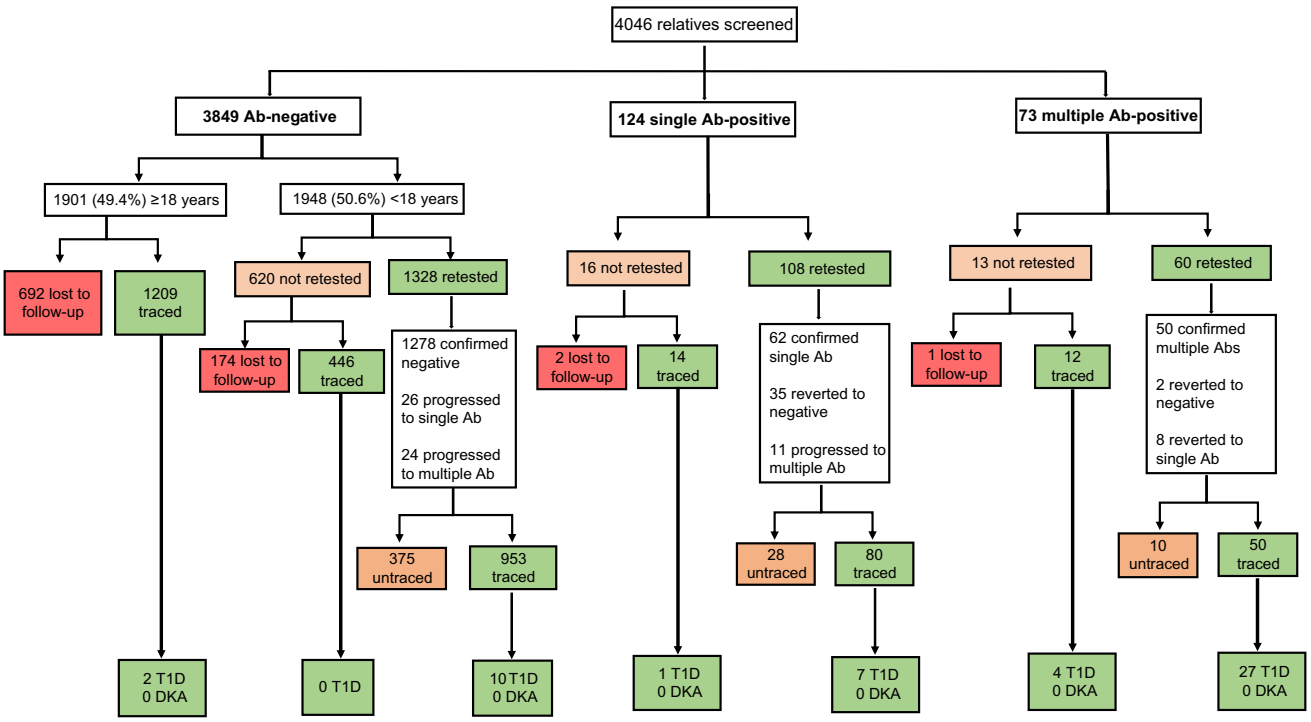

Fig. 1 Participants' flow diagram. Ab, autoantibody; T1D, stage 3 type 1 diabetes

age, and nearly half were aged 18 years or over. At the initial screening, 4.9% of relatives were identified as autoantibody positive, with 3.1% being positive for a single autoantibody and 1.8% being positive for multiple autoantibodies. The proportion of participants who tested positive for at least one autoantibody was consistent across the three age groups: 4.5% under 10 years, 5.4% between 10 and 17 years, and 4.8% over 18 years (p=0.72). In autoantibody-positive participants, the most frequent autoantibody was GADA (3.9% prevalence), followed by IAA (1.4%) and IA-2A (1.0%).

Across age groups, no significant difference was found in the distribution of GADA. Conversely, IAA were more frequently found in positive participants younger than 10 years, while IA-2A were more commonly detected among those aged 10–17 years.

Follow-up and progression to stage 3 type 1 diabetes A detailed flow diagram of the study cohort showing follow-up and outcomes is depicted in Fig. 1. Overall, follow-up information was available for 3177/4046 (78.5%) participants, with a median (IQR) follow-up duration of 9.9 (6.5–13.5) years (range 0.1–18.7 years). Among the 869 (21.5%) participants who were lost to follow-up, 692 were not re-screened after the initial visit, in accordance with the protocol, as they were autoantibody-negative adults. The remaining 177 participants who missed their scheduled re-screening had a median (IQR) age of 9.0 (5.0–13.0) years and the majority (174) were autoantibody negative, while two were positive for a single autoantibody and one was positive for multiple autoantibodies.

Regular follow-up visits with autoantibody retesting and, in positive individuals, metabolic monitoring, were performed for 1328/1948 (68.2%) of the initially autoantibody-negative participants, 108/124 (87.1%) single-autoantibody-positive participants and 60/73 (82.2%) multiple-autoantibody-positive participants. A total of 413 participants (10.2% of the study population) attended at least one re-screening visit but were not subsequently re-traced. The median (IQR) age of these participants was 8.0 (3.0–13.0) years. Of these participants, 375 tested negative, 28 had a single-positive result, and ten had a multiple-positive result at first screening.

Progression to stage 3 type 1 diabetes occurred in 51/3177 (1.6%) of all relatives. Of these, 12/2983 (0.4%) were initially autoantibody negative, 8/122 (6.5%) had a single autoantibody and 31/72 (43.1%) had multiple autoantibodies. Within the group of participants who were positive for multiple autoantibodies at first screening, a significantly higher proportion of those initially screened as children progressed to stage 3 type 1 diabetes as compared with those screened as adults (53.2% vs 24.0%) (p<0.001). The incidence rate for stage 3 type 1 diabetes was 0.3 (95% CI 0.2, 0.5), 7.1 (95% CI 3.6, 14.2) and 61.8 (95% CI 43.4, 87.8) per 1000 person-years (p<0.001) for participants positive for 0, 1 and ≥2 autoantibodies, respectively.

Figure 2 shows the Kaplan–Meier survival analysis. The projected survival free of stage 3 type 1 diabetes at 15 years of follow-up was 99.5% (95% CI 99.1, 99.7) in autoantibody-negative participants, 87.3% (95% CI 74.4, 94.0) in participants with a single autoantibody and 45.9% (95% CI 31.1, 59.6) in participants with multiple autoantibodies (p<0.001).

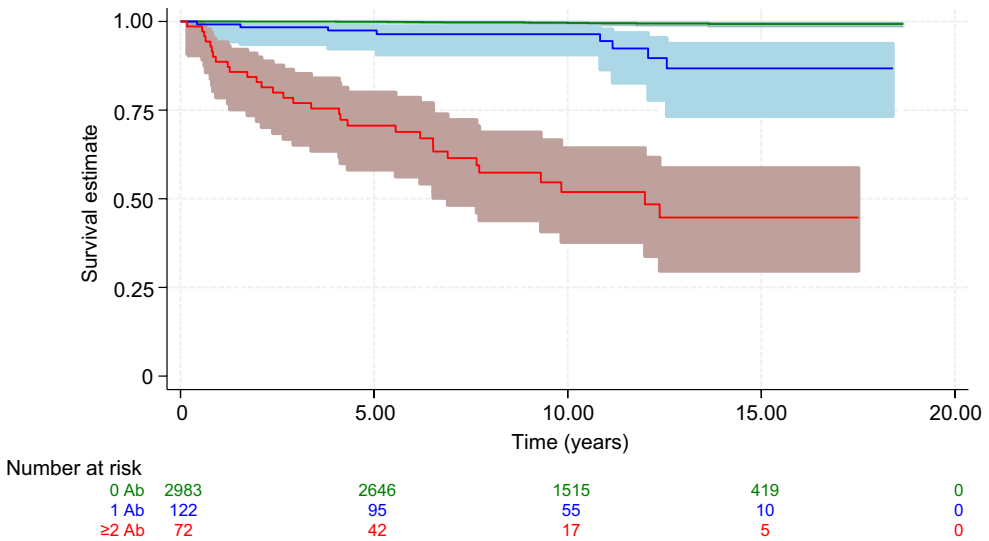

Fig. 2 Survival free of stage 3 type 1 diabetes after the first autoantibody screening test. Green line, autoantibody-negative; blue line, positive for a single autoantibody; red, positive for ≥2 autoantibodies. CIs are displayed around the survival curves. Statistical test used for comparison: logrank test (p value <0.001). Ab, autoantibody

Table 2 Characteristics of participants who progressed to stage 3 type 1 diabetes (*N*=51)

Characteristic	Overall	No. of autoantibodies reported at first screening			*p* value
		0	1	≥2	
Type 1 diabetes events, *n*	51	12	8	31	
Female sex, n (%)	23 (45.1)	5 (41.7)	3 (37.5)	15 (48.4)	0.83
Time to onset (years), median (IQR)	5.1 (1.8–8.9)	7.8 (5.4–10.4)*	7.9 (2.1–11.8)	2.9 (0.9–6.5)*	0.01
Age (years) at onset, median (IQR)	14.5 (10.1–23.9)	14.1 (10.6–20.8)	19.3 (5.9–32.0)	14.5 (9.8–24.0)	0.99
Adults at onset, *n* (%)	16 (31.4)	4 (33.3)	4 (50.0)	8 (25.8)	0.42
Adults at first screening, *n* (%)	10 (19.6)	2 (16.7)	2 (25.0)	6 (19.4)	0.90
OGTT at last visit, *n* (%)[a]	38 (74.5)	10 (83.3)	3 (37.5)	25 (80.6)	0.46
Normal OGTT	16 (42.1)	6 (60.0)	2 (66.7)	8 (32.0)	
IGT	13 (34.2)	2 (20.0)	1 (33.3)	10 (40.0)	
Diabetic	9 (23.7)	2 (20.0)	0 (0.0)	7 (28.0)	
HbA_{1c} (mmol/mol) at onset, median (IQR)[b]	64 (52–86)	79 (52–86)	74 (61–86)	62 (52–69)	0.78
HbA_{1c} (%) at onset, median (IQR)[b]	8.0 (6.9–10.0)	9.4 (6.9–10.0)	8.9 (7.7–10.0)	7.8 (6.9–8.5)	0.78
Type 1 diabetes-related hospitalisations, *n* (%)	22 (43.1)	6 (50.0)	3 (37.5)	13 (41.9)	0.84
DKA, *n* (%)	0 (0.0)				
Venous pH at onset, median (IQR)[c]	7.37 (7.35–7.39)	7.38 (7.38–7.39)	7.39 (7.30–7.40)	7.36 (7.35–7.37)	
ICU, *n* (%)	0 (0.0)				

[a]Data missing for 13 individuals

[b]Data missing for 17 individuals

[c]Data missing for 12 individuals

The *p* values related to between-three-groups comparison are derived from the Kruskal–Wallis test for continuous variables and the χ^2 test for categorical variables; *$p<0.05$ between groups, identified through post hoc pairwise comparisons ICU, intensive care unit; IGT, impaired glucose tolerance

The median (IQR) time to diagnosis was 7.8 (5.4–10.4) years in autoantibody-negative participants, 7.9 (2.1–11.8) years in single-autoantibody-positive participants and 2.9 (0.9–6.5) years in multiple-autoantibody-positive participants (p=0.012).

In 16 participants, type 1 diabetes was diagnosed in adult age, and ten of them were aged ≥18 years at screening. Among the group of 1328 participants aged <18 years who were initially autoantibody negative and were retested during follow-up, 50 seroconverted at a median (IQR) age of 8.5 (5.0–11.0) years; 17 (34%) of these participants seroconverted after the age of 10 years. It was observed that all individuals who progressed to stage 3 type 1 diabetes had become multiple-autoantibody positive in the meantime. Among the group of 1209 participants aged ≥18 years who were initially autoantibody negative and subsequently traced in 2023–2024, two individuals progressed to stage 3 type 1 diabetes at the age of 24 and 40 years, 6.0 and 13.6 years after screening, respectively. At the time of type 1 diabetes diagnosis, one of these two participants was positive for ZnT8A (not tested in primary screening) while the other was positive for GADA and ICA.

DKA and clinical characteristics at stage 3 type 1 diabetes diagnosis The characteristics of the 51 participants who progressed to stage 3 type 1 diabetes are reported in Table 2. Most of the participants underwent repeated OGTT and HbA_{1c} measurements during the follow-up period. The last visit was conducted a median (IQR) of 1.3 (0.4–3.3) years before disease onset. In nine participants, stage 3 type 1 diabetes diagnosis was made in concomitance with the last OGTT. There were no cases of DKA. Hospitalisation for diabetes education and insulin therapy is standard practice for new-onset paediatric patients at San Raffaele Hospital, regardless of DKA. Accordingly, 22 paediatric participants (43.1%) were hospitalised at the time of clinical diagnosis, with none requiring admission to the intensive care unit. The median (IQR) age of hospitalised patients was significantly lower than those not hospitalised: 10.1 (7.5–13.1) vs 17.3 (14.1–26.8) years, respectively (p<0.001). Blood gas analysis was evaluated in ten hospitalised participants, showing a pH value in the normal range.

Discussion

This report highlights the experience of one site within the international, multisite TrialNet Pathway to Prevention study, confirming that islet autoantibody screening within

families with type 1 diabetes allows the identification of people in the pre-symptomatic phase of the disease and has the potential to fully prevent DKA at the time of clinical onset.

The reduction of DKA incidence in stage 3 type 1 diabetes after autoantibody screening has been reported in several prospective studies conducted in families, genetically selected cohorts and, more recently, in the general population, with rates dropping to as low as 2.5% in some studies [7–13]. Our work is the first to show that no DKA was detected in any of the screened participants who progressed to stage 3 type 1 diabetes. This further reduction is likely attributable to all educational activities and follow-up interactions with families being conducted at a single centre.

The incidence of DKA was 32.6% in 928 children newly diagnosed with type 1 diabetes from the general population with no prior screening referred to the Paediatric Endocrinology Unit at San Raffaele Hospital during the last 11 years (2013–2023). Although a lower incidence of DKA has been reported among unscreened relatives of individuals with type 1 diabetes when compared with the general population [15], this information is not available for the Italian population. Therefore, our results reinforce the value of autoantibody screening as a vital tool for the identification of pre-symptomatic type 1 diabetes and DKA prevention at disease onset [7–13].

The key to DKA prevention is the early diagnosis of pre-symptomatic type 1 diabetes and the timely initiation of insulin therapy [24]. These goals can be achieved through the implementation of an educational intervention, metabolic staging and periodic monitoring. In our study, these components were part of the protocol [20, 21]; a significant number of participants remained in contact with the centre for an extended period of time. In recent works, the same procedures have been incorporated as recommendations and reference guidance for islet-autoantibody-positive individuals with pre-symptomatic type 1 diabetes [25–27]. The implementation of structured protocols is an effective method for DKA prevention. However, this is contingent upon the reliability and consistency of interaction between specialists at the diabetes centre and people and families during follow-up. In our cohort, progression to stage 3 type 1 diabetes confirms the strong association with the number of islet autoantibodies, as indicated by the Kaplan–Meier estimated risk at 15 years after the initial screening. Our findings are consistent with those reported by other prospective studies [3, 28]. Notably, the risk associated with single autoantibody positivity is confirmed to be low, but not negligible; single positivity often precedes a late conversion to multiple autoantibodies in those who further progress to stage 3 type 1 diabetes.

An interesting implication of our results relates to the optimal age for screening in order to identify the largest number of individuals at risk for type 1 diabetes. Two recent reports, including numerous prospective studies conducted globally, suggested the ages of 2 and 6 years for initial screening to predict childhood type 1 diabetes [29] and 10 years to predict type 1 diabetes up to the age of 18 years [30]. Our study cohort spanned a wide age range, with nearly half of the participants being aged 18 years or above. In line with literature, we found a significant association between autoantibody groups and age groups, with a higher proportion of adults in those who tested single positive compared with multiple positive.

With respect to seroconversion over time, one-third of those initially autoantibody negative seroconverted after the age of 10 years. Furthermore, the two individuals who initially screened negative at ≥18 years of age and subsequently developed stage 3 type 1 diabetes were both autoantibody positive at the time of diagnosis. In light of these findings, an additional opportunity for autoantibody testing might be offered beyond the age of 10 years, potentially around 18 years, with the aim of maximising the identification of individuals at risk.

The homogeneity of the screening procedures and the accuracy of the follow-up and monitoring represent a key strength of the study. These were carried out over an extended period of time at the same centre by the same team, ensuring consistency and reliability. This approach ensured strict adherence to the Pathway to Prevention study protocol throughout its duration and facilitated the establishment of positive relationships with the families involved. Furthermore, the most recent update on the cohort of participants was conducted via personal contacts, with most interactions conducted via telephone. In many cases, these interactions were preceded by e-mails, messages or other forms of communication. This process was overseen by two dedicated physicians. This approach permitted the status of 78.5% of those screened to be traced and updated, including those who were initially antibody negative and had no subsequent follow-up.

The main limitation of our study is its single-centre design and the relatively small group of individuals who developed stage 3 type 1 diabetes, thereby reducing the generalisability of our findings. Indeed, the relatively modest sample size of the cohort is comparable with that of other family studies, although it is smaller than the sample size reached in existing screening programmes in the general population, such as Fr1da [31] or ASK [32]. Nevertheless, our study was conducted at a single site within TrialNet, the largest programme conducting screening in relatives worldwide [21], whereby the experience at a single site can be shared with the many other sites of the consortium.

Another notable limitation is the presence of missing data at follow-up, with 21.5% of study participants lost to follow-up. However, most of them were not re-screened

after the first visit as they were antibody negative at the age of ≥18 years, in accordance with the study protocol. Likewise, those aged <18 years who missed the scheduled re-screening were mostly antibody negative, possibly indicating a low perceived risk in these relatives that could explain their non-participation in the re-screening. Additionally, 413 participants underwent at least one re-screening but could not be traced in 2023–2024. However, it is unlikely that these individuals had an impact on the study's findings, as they were similar in terms of age and antibody distribution to those who were successfully traced.

The key finding of the study is the potential of full DKA prevention at stage 3 type 1 diabetes clinical onset in a population of relatives. Outside research studies, a regular and periodic follow-up of autoantibody status and metabolism variables is challenging. However, raising awareness among both individuals at increased risk and healthcare professionals is essential to facilitate the translation of these findings into clinical practice. The education of individuals in pre-symptomatic type 1 diabetes, the promotion of periodic autoantibody and metabolic monitoring, and the provision of adequate psychosocial support are complex but fundamental steps in this process, as summarised in the recommendations of a recently published Consensus Guidance [26]. There were no potential implications of sex/gender on the study results and analyses.

In conclusion, the TrialNet Pathway to Prevention experience at one single centre confirms that autoantibody screening, followed by accurate clinical monitoring including education and metabolic staging, is an effective strategy for significantly reducing, and potentially completely preventing the occurrence of DKA at the time of stage 3 type 1 diabetes clinical diagnosis in a cohort of relatives of individuals with type 1 diabetes. The extent to which these results can be replicated in general population screening programmes remains to be determined.

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Data availability The data that support the findings of this study are available from the corresponding author. Restrictions apply to the availability of these data, which were used under license from the TrialNet (USA) for the current study and so are not publicly available. Data are, however, available from the corresponding author upon reasonable request and with permission from the TrialNet Consortium.

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Authors' relationships and activities The authors declare that there are no relationships or activities that might bias, or be perceived to bias, their work.

Contribution statement EBo was a member of the TrialNet Study Group and had clinical and scientific responsibility for the conduction of TrialNet studies at the centre. PG was the Trial Coordinator. SM, MRP, RB were the physicians responsible for the contact with participants and families for screening and follow-up. EBi, PG, FR and EBa were responsible for the conduction of screening, follow-up and monitoring procedures, including periodic contact, administration of educational tools, execution of immunological and metabolic tests. ES, AG and FM were responsible for the phone and e-mail contacts during 2023 and 2024 with participants for whom there was no further contact after the first screening and those with no updated information during follow-up. SM, AM, ES and FM designed and conducted the statistical analysis. EBo, SM and AM wrote the manuscript. All authors contributed to discussion and reviewed/edited the manuscript and gave final approval for the paper to be published. EBo is responsible for the integrity of the work as a whole.

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