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Population-Based Screening for Type 1 Diabetes in Czechia: Results From the First 1% of the Paediatric Population

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ABSTRACT

Aims: EDENT1FI is a European initiative aiming to implement a roadmap for screening, monitoring and prevention of childhood Type 1 diabetes (T1D). The Betty project is a nationwide T1D screening program recently established in Czechia within the framework of EDENT1FI. Here we report its results after having tested 1% of the Czech paediatric population.

Methods: The screening targets children aged 2–17.9 years, regardless of whether they have a family history of T1D. It follows a two-step process for detecting islet-specific autoantibodies (anti-GAD, anti-IA2, anti-ZnT8 and IAA). If the capillary screening sample is positive in the first-level test (screening ELISA yielding a summary signal of three autoantibodies), the same sample is retested at the EDENT1FI reference laboratory using a battery of 4 second-level tests for individual autoantibodies (LIPS or RBA). When any autoantibody is detected, a venous sample is requested for confirmation. If multiple autoantibodies are confirmed from this venous sample, the child is diagnosed with early-stage T1D and offered follow-up tailored to their individual estimated rate of progressing to clinical diabetes. Children with only a single confirmed autoantibody are offered a less intensive follow-up outside the EDENT1FI protocol.

Results: The Betty project collected 19 044 capillary screening samples (1% of the paediatric population) between April 2024 and February 2026. Samples were collected across 270 screening sites mostly at primary care and via home sampling (4.1% samples). The majority of participants (89.7%) were from the general population having no first-degree relative (FDR) with T1D. We found a prevalence of multiple autoantibodies of 0.39% (95% CI: 0.30%–0.51%) in the general population and 2.8% (95% CI: 2.1%–3.7%) prevalence among FDRs of subjects with T1D. At the time of writing, of 201 children with non-negative capillary screening sample, 102 were diagnosed with early-stage T1D by a confirmatory venous sample and 83 had their T1D stage assessed: 77 (93%) were in Stage 1, 4 (4.8%) in Stage 2 and 2 (2.4%) in Stage 3. Further seven children, who were identified by capillary screening,

The list of collaborators is attached as Supporting Information.

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manifested with Stage 3 T1D before their staging could be completed. All families who came for staging and education visits consented to long-term follow-up.

Conclusions: This nationwide T1D screening program successfully reached approximately 1% of the Czech paediatric population, demonstrating the feasibility of implementing population-based islet autoantibody screening based on the common EDENT1FI protocol. These findings provide an important foundation for the potential future implementation of routine population-based T1D screening within the Czech national healthcare system and offer valuable insights for other countries considering similar screening programmes.

1 | Introduction

Type 1 diabetes (T1D) is an autoimmune disease with early asymptomatic stages marked by the presence of multiple autoantibodies against antigens of the pancreatic islet beta cells [1, 2] a reliable tool for identifying individuals with future clinical T1D [3]. Presently, between 30% and 50% of children with T1D are diagnosed in a state of diabetic ketoacidosis [4], a preventable complication with both acute and long-term consequences [5–7]. Detecting T1D in its early presymptomatic stages enables timely education and the initiation of insulin therapy, thus significantly lowering the rate of DKA at onset [8, 9]. It also provides opportunities for the use of preventative immunotherapies [10]. This has become even more clinically important in light of the recent success of teplizumab, which is now approved in a growing number of countries worldwide for delaying the onset of T1D in children and adults in Stage 2 of T1D [11].

A number of projects worldwide employed different screening approaches to identify individuals with multiple islet-autoantibodies. Some were targeting a high-risk population defined based either on family history [12–14] or HLA-associated risk [15]. Others tested T1D-associated autoantibodies among the general paediatric population with or without defined age limits [3, 16]. Together, these programmes have demonstrated that screening for early-stage T1D is a feasible approach to improving outcomes across all stages of the disease and provides strong support for the implementation of population-based islet autoantibody screening [17–19]. However, the success of such programmes ultimately depends on their accessibility, robust infrastructure, public engagement and the willingness of families to participate in confirmatory testing and subsequent follow-up after a positive screening result [20–22].

The *European action for the Diagnosis of Early Non-clinical Type 1 diabetes For disease Interception* (EDENT1FI) is a global collaborative project of 27 partners in 13 countries which aims to establish a roadmap for the screening and prevention of T1D in children. Its goal is to screen 200 000 children across Europe by testing the presence of multiple islet autoantibodies in a capillary sample and to include the ones with early-stage T1D in a long-term follow-up [23]. The EDENT1FI screening protocol was based on the experience from the German *Fr1da* study [3].

The *Betty* project is a Czech branch of this international framework. In this paper we present the feasibility and results of the study after screening 1% of the national paediatric population.

2 | Subjects, Materials and Methods

The EDENT1FI consortium was established in November 2023. In its framework, we launched the nationwide population screening (the *Betty* project) in April 2024. This report covers the period until 13 February 2026.

The study protocol is based on a two-tier testing of specific autoantibodies adapted from the German Fr1da-study [3] and is described in the EDENT1FI Master Protocol for Screening [23].

2.1 | Study Population and Screening Sites

Screening within the *Betty* project was aimed at children aged 2–17.9 years without previous diagnosis of clinical T1D, regardless of their family history of T1D. Samples were collected by a nationwide network of screening sites—primary paediatricians and paediatric diabetologists who volunteered to participate in the project.

The project was presented to paediatricians through an information campaign consisting of lectures at national conferences for Paediatrics and Diabetology, online webinars and articles published in both national paediatric journals and the media (television, radio, social networks). As all children in Czechia are registered with a primary care paediatrician who conducts regular preventive health visits every 2 years, primary care paediatricians were the main target of this campaign. Paediatric diabetologists were also invited to participate. Health care providers interested in the project contacted the Project Coordination Office at University Hospital Motol (UH Motol) by email or the project website to register as a screening site. Screening sites then received all necessary materials (test tubes, lancets, laboratory request forms, informed consent forms, leaflets, etc.) free of charge from the project coordination office. They were also reimbursed for screening-associated administrative work, €5 for each screened child.

The screening centres themselves offered the screening to families. After a thorough explanation of the rationale behind the project by the sampling physician, an informed consent form signed by one of the parents/caregivers was collected from every participating child. A request form accompanying the capillary screening sample contained information about name, date of birth, height, weight, postcode, presence of T1D in first-degree relatives (FDRs), name of the parent/caregiver and their contact telephone and/or email.

A possibility of home sampling was also offered through the project's website, aimed at families with limited access to

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Highlights

- Detection of T1D in its early stages allows a timely start of insulin therapy, significantly lowers the rate of DKA at onset and provides opportunities for the use of preventative immunotherapies.
- *The European action for the Diagnosis of Early Non-clinical Type 1 diabetes For disease Interception* (EDENT1FI) is a global collaborative initiative aiming to establish a roadmap for the screening and prevention of T1D in children in Europe.
- To what extent can a population-based screening program based on a shared international protocol be successfully implemented in a country with no prior experience?
- The *Betty* project is a local branch of the EDENT1FI framework which launched a population T1D screening in Czechia in April 2024.
- Within less than 2 years, the screening expanded nationwide, covering 1% of the target paediatric population and identifying a 0.39% prevalence of multiple autoantibodies in the general population and 2.8% among first-degree relatives of subjects with T1D.
- Results of the *Betty* project demonstrate the feasibility of implementing population-based islet autoantibody screening based on the common EDENT1FI protocol and provide a foundation for the potential future implementation of routine T1D screening within the national healthcare system.

the established screening sites. Families that were unable to have the sample collected at an established screening centre could request a home sampling kit through the project's website or by email. The home sampling kit was then sent to the family by post from the project coordination office. Explanation of the project and instructions for sample collection were provided by email, in a printed information sheet and in a video.

2.2 | Sample and Data Flow

From each participant, a 100–200 μL capillary blood screening sample was taken into an EDTA-anticoagulated vial with serum separator (Sarstedt, Hildesheim, Germany) labelled with a 2D barcode that was printed also on the enclosed request form. The sample was stored locally in the refrigerator before its weekly postal shipment to the central laboratory at UH Motol. There, the patient's data were entered into the project database, and the serum was separated and stored at −80°C in a 2D barcoded microtube organised in 96-position racks (Micronics, Lelystad, the Netherlands). Haemolysis of the separated serum was visually inspected on and/or quantified spectrophotometrically at 410 nm [24].

The first-tier screening was performed using a combined enzyme linked immunosorbent assay (3 Screen ICA ELISA kit, RSR Ltd., Lancaster, UK) yielding a summary signal from three autoantibodies: islet antigen-2 autoantibodies (anti-IA2), glutamic acid decarboxylase autoantibodies (anti-GAD) and zinc transporter-8 autoantibodies (anti-ZnT8). The laboratory participated in the last (2024) Islet Autoantibody Standardisation Programme [25] with a 96.0% sensitivity and 94.8% specificity.

Negative screening results in this first-tier ELISA were communicated without delay by e-mail to the caregivers and in cumulative reports every 2 months also to the screening site.

If the first-tier ELISA yielded a positive summary signal result (> 25 units/mL), or if the ELISA signal was increased significantly compared to the background reactivity of the test plate, the remaining capillary serum was sent for further testing to the EDENT1FI project's reference laboratory at the Institute for Diabetes Research, Helmholtz Munich, in Munich, Germany. There, a battery of four individual tests was performed: anti-IA2, anti-GAD, anti-insulin autoantibodies (IAA) as well as anti-ZnT8 (in both allelic variants) were measured separately using radiobinding assay (RBA) or luciferase immunoprecipitation system (LIPS) assay.

In these retested samples, the communication with caregivers and the screening centre was deferred until the completion of the second-tier tests. Negative results were communicated in the same manner as if concluded by the first-tier testing: by e-mails to both the caregivers and the screening site.

If the referred capillary screening sample was positive for multiple islet autoantibodies, indicating a high probability of the future diagnosis of early-stage T1D, the screening site was notified by email and the family was contacted by phone to request a venous confirmatory sample and to be provided with basic information on symptoms present at onset of clinical T1D. Parents were encouraged to proceed without delay with the venous confirmation. Venous confirmation samples were also requested when the capillary sample could not be clearly evaluated at the reference laboratory due to a limited sample volume.

Venous confirmatory samples were collected at the screening sites and sent by post. Serum was separated and stored at UH Motol and one of the aliquots was sent to the reference laboratory in Munich where LIPS and RBA were performed as described above.

2.3 | Early-Stage T1D Diagnosis, Staging and Follow-Up

Early-stage T1D was defined according to the EDENT1FI approach ('rule of twos'): positivity for two or more distinct islet autoantibodies confirmed in an independently collected venous blood sample and using a different assay format from the screening assay [23]. Children, whose positivity for two or more islet autoantibodies was thus confirmed by the venous sample, were diagnosed with early-stage T1D. The diagnosis was communicated to the parents and to the screening site by one of the staff physicians of the national coordinator. First, one of the parents was contacted by phone to explain the result and provide basic information about T1D, its stages and symptoms. The family was then invited for an education and staging visit.

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The T1D stage was determined by fasting glycaemia, oral glucose tolerance test and HbA1c (criteria are listed in Tables S1, S2). The individual risk of progression to Stage 3 T1D was assessed using the progression likelihood score (PLS), which is based on the anti-IA2 titre, HbA1c and glycaemia at 90 min during oGTT [26]. Further follow-up was stratified with the frequency of visits (every 3, 6 or 12 months) based on age, T1D stage and PLS according to the EDENT1Follow Master protocol. All visits were held at the national coordination site at UH Motol. Those who did not wish to proceed per protocol at UH Motol were offered a minimal contact alternative aimed mostly at DKA prevention.

2.4 | Children Having a Single Autoantibody

On top of the standard EDENT1FI protocol, we decided to also follow those being positive for one islet autoantibody only. In case of a single autoantibody presence in the capillary screening sample, the screening site and the family were informed about the potential increased risk of future T1D and a venous confirmatory sample was requested. Children with confirmed single autoantibody presence were offered a follow-up consisting of yearly measurements of autoantibodies and HbA1c as suggested by Phillip et al. [27].

2.5 | Statistical Analysis

Prevalence figures were calculated from completed tests (i.e., the denominators excluded pending results and exhausted samples) and were reported along with binomial proportion confidence intervals according to Wilson. Associations were quantified using odds ratios calculated from 2 × 2 tables, together with corresponding confidence intervals. The Kaplan–Meier estimator was used to evaluate the time interval between communicating the positive capillary screening result and collecting the venous confirmatory sample.

3 | Results

3.1 | Participation and Screening Sites

A total of 19 044 capillary screening samples were collected, representing slightly over 1% of the target Czech children population aged 2–17.9 years [28]. Of these, 18 268 samples (95.9%) were collected by screening sites and 776 samples (4.1%) by parents through home sampling. Immediately after admission, 618 (3.2%) samples had to be rejected, mostly due to bacterial decay of the sample evidenced by severe haemolysis and serum that could not be separated, or due to drying out of a tube that had not been properly sealed. Samples collected by parents and posted individually were no less likely to be rejected ($p = 0.11$) than samples sent in batches by screening sites. Further 62 samples were excluded from the present analysis as they were taken by mistake very early after a previous negative screening result.

The study network has grown to a total of 270 actively recruiting screening sites distributed across the whole country (Figure 1). The count of capillary screening samples per screening site varied widely, with the top five sites contributing 799, 568, 552, 551 and 495 samples, while 66 sites collected less than 10 samples. The cumulative proportion of samples per site is shown in Figure S1. The 50 most active sites contributed 66.7% of samples and a further 50 added another 18.9%.

3.2 | The Children in the Study

Characteristics of the 17 876 children, whose capillary screening result was concluded at the time of writing, are presented in Table 1. The flow of samples and their results through the steps of the screening process is presented in Figure 2.

The median age of screened children was 9.4 (IQR 5.8–13.3) years; 50.5% were female. Figure S2 shows the monthly counts of the capillary screening samples itemised to 1-year age bands. Odd years of age are slightly overrepresented as compared to even years, probably due to biannual mandatory paediatric check-ups.

Of the 17 876 children with a concluded capillary screening sample, 16 027 (89.7%) were from the general paediatric population, having reported no FDRs with T1D. Subjects with an FDR with T1D ($n = 1849$, 10.3%) did not differ in age or sex distribution but were significantly more likely to provide self-collected samples (19.5% vs. 2.0% from the general population, $p < 10^{-4}$).

3.3 | Capillary Screening Results and Multiple Autoantibody Prevalence

Two or more islet autoantibodies were detected in 115 of 17 876 children (0.64%) whose capillary screening result was concluded at the time of writing (Figure 2A). In the general paediatric population, the prevalence of multiple islet autoantibodies was 0.39% (95% CI: 0.30%–0.51%; 63/16027 subjects), as compared to 2.8% (95% CI: 2.1%–3.7%; 52/1849 subjects) among children having an FDR with T1D (Table 2). Therefore, having an FDR with T1D was associated with an odds ratio of 7.3 for testing positive for multiple islet autoantibodies (95% CI: 5.0–11, $p < 10^{-5}$).

A single islet autoantibody was detected in 75 (0.42%) children (51/16027 = 0.32% in the general population vs. 24/1849 = 1.30% in the FDR), thus having FDR with T1D was associated with an odds ratio of 4.1 for testing positive for a single autoantibody (95% CI: 2.5–6.7, $p < 10^{-5}$).

Capillary samples of a further 11 (0.06%) children were inconclusive, being positive in the screening ELISA, but in the reference laboratory the sample was exhausted after one autoantibody was detected.

The remaining 17 675 (98.9%) subjects were free of islet autoantibodies in the capillary screening sample. Most of them (17 222, 96.3%) could be concluded already by the initial screening first-tier ELISA test, whereas 453 (2.53%) capillary screening samples had to be reassessed by a battery of individual autoantibody tests in the central reference laboratory before being concluded as negative and reported to the caregivers and the screening site.

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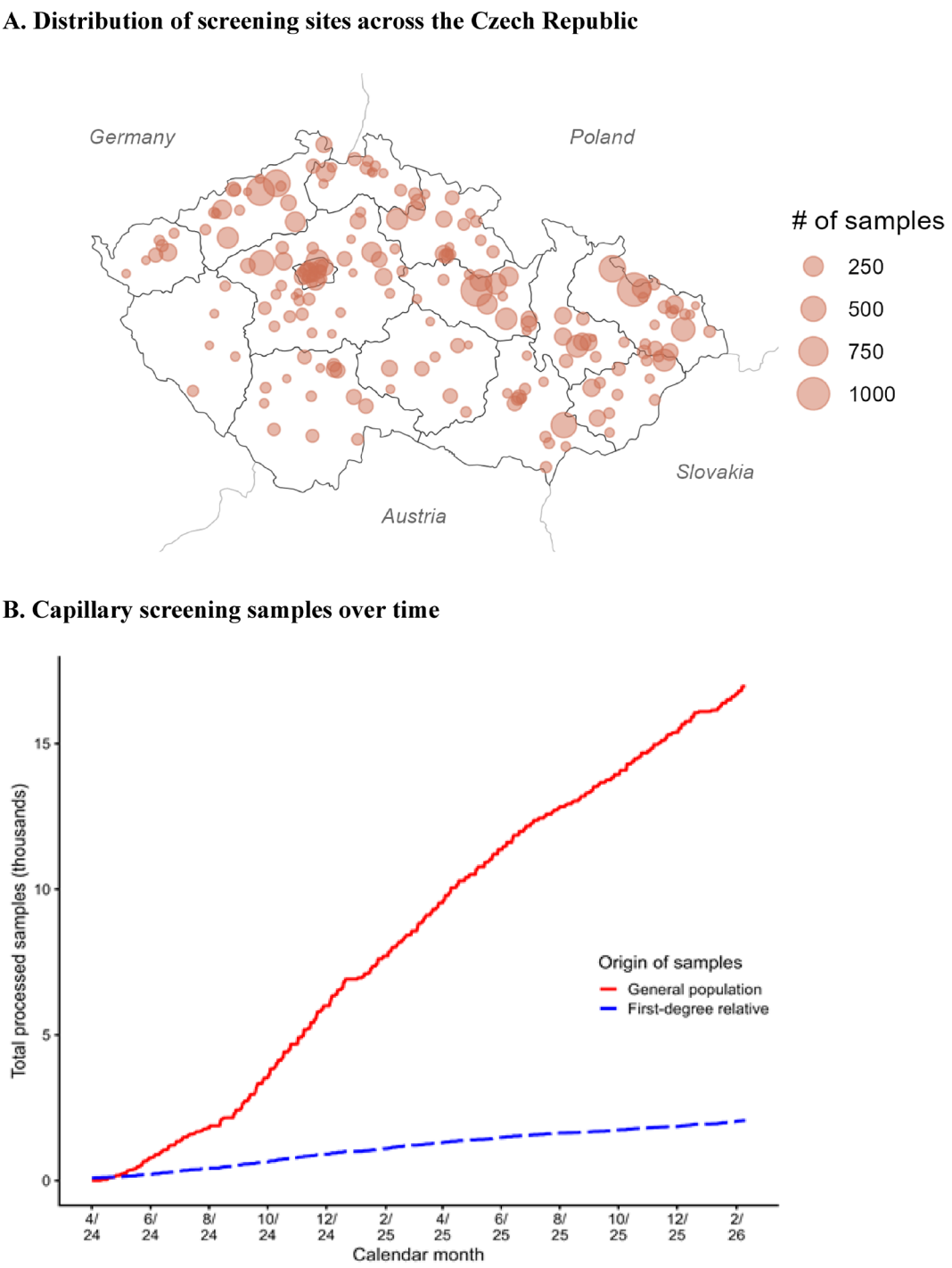


FIGURE 1 | Screening sites and capillary screening sample count.

A simple post hoc exploratory analysis indicated that the potential positive predictive value of the first-tier screening ELISA assay could be higher, subject to prospective validation, if the manufacturer's positivity threshold (25 arbitrary U/mL) was increased for use in the general population, without compromising sensitivity to detect subjects with multiple islet autoantibodies (Table S2).

We also assessed whether the positive ELISA result of the capillary screening sample might be associated with the quality of the sample. Chi square test for trend showed no effect of haemolysis degree on the positivity proportion ($p = 0.93$) as shown in Figure S3.

3.4 | The Confirmation Process Using Venous Confirmatory Samples and the Prevalence of Early-Stage T1D

A venous confirmatory sample was requested from all 201 children whose capillary screening samples could not be unequivocally concluded as negative. At this point, two families rejected

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TABLE 1 | Characteristics of children with concluded capillary screening samples.

	General population[a]	Subjects having an FDR with T1D	Total subjects
n (% of total)	16027 (89.7%)	1849 (10.3%)	17876
Sampling self-administered by parent	321 (2.0%)	352 (19.5%)	673 (3.8%)
Female sex	8070 (50.5%)	941 (50.9%)	9011 (50.6%)
Age (median, IQR)	9.4 (5.8–13.3)	9.3 (5.6–13.3)	9.4 (5.8–13.3)
Relatives with T1D			
Has mother with T1D		569 (31.3%)	
Has father with T1D		614 (33.7%)	
Has sibling(s) with T1D		741 (41.7%)	
Of these, more than one FDR		75 (4.1%)	

Note: Only subjects with a concluded capillary testing are counted: their capillary screening samples are concluded by negative screening ELISA or underwent testing in the reference laboratory. These are outlined by the orange dashed box in Figure 2A and correspond to Figure S6, boxes 'ELISA negative', 'Reference laboratory: no autoantibodies detected in capillary sample', 'Reference laboratory: 2+ autoantibodies detected', 'Reference laboratory: a single autoantibody detected' and 'Reference laboratory: inconclusive pattern in capillary sample'.
[a]Subjects having no known first-degree relative with T1D.

further collaboration despite their initial consent, and two subjects manifested with Stage 3 T1D before delivering the venous confirmatory sample (Figure 2B).

Of the 163 children for whom the results of the venous confirmatory sample were available, 102 had multiple autoantibodies and were thus concluded as having early-stage T1D, 52 had a single autoantibody and 9 were negative. The breakdown of the trajectories of antibody patterns from the capillary screening results to the venous confirmatory antibody patterns is shown in Figure 3 and Table S3. Most subjects retained their antibody positivity pattern, except for three who reverted from multiple autoantibodies in the capillary sample to a single autoantibody positivity in the venous confirmatory sample and six who converted from a single autoantibody to multiple autoantibodies. Nine of 60 (15%) subjects with a single autoantibody in the capillary screening sample reverted to complete negativity in the venous sample.

3.5 | Staging and Follow-Up of Children Diagnosed With Early-Stage T1D

All 102 children diagnosed with early-stage T1D were invited for the education and staging visit and at the time of writing, 83 children had it completed (Figure 2C). Two families (2.0%) refused to come for the staging and education visit. Of the 83 children with concluded T1D staging, 77 children were in Stage 1 (92.8%), 4 children in Stage 2 (4.8%) and 2 children in Stage 3 (2.4%). Four children did not complete the oGTT, and their stage was therefore based solely on HbA1c.

All families who completed the education and staging visit consented to participate in long-term follow-up. Of these, 76 families (91.6%) consented to follow-up according to the full EDENT1Follow protocol and 7 families (8.4%) opted for minimal contact follow-up with visits held closer to their home, which are limited to the clinical assessment and no samples are taken for research purposes.

3.6 | The Process Flow

The time span between important process nodes is shown in Figure S4. The time from blood collection to the admission in the laboratory was 8 [5–11] days (median [IQR]). Time from admission to e-mailing a negative result was shorter if the negative result was made already by first-tier ELISA (20 [14–28] days) than if the capillary screening sample had to be retested by the battery of individual autoantibody tests in the reference laboratory (77 [57–105] days). In capillary screening samples with positivity for two or more islet autoantibodies, it took 65 [55–84] days from sample admission to communicating the result to the parents and to the screening site. The median time from communicating the positive result of the capillary screening sample to collecting the venous confirmatory sample was 32 days (95% CI: 26–35 from a Kaplan–Meier estimator), Figure S5. A detailed-oriented diagram of sample flow in the process is presented in Figure S6.

4 | Discussion

Results from the *Betty* project show that the approach and methodology applied to implement a population T1D screening programme are feasible and effective. In less than 2 years, T1D screening spread nationwide and reached a milestone of testing 1% of the country's paediatric population. These results document the rapid and successful adoption of the international EDENT1FI protocol.

The study detected multiple autoantibody positivity of the capillary screening sample in 0.39% subjects among the general paediatric population and in 2.8% subjects with an FDR with T1D. These results are in line with proportions found by other T1D screening programmes worldwide [3, 13, 19].

Of the children with confirmed early-stage T1D, the vast majority were in Stage 1 (93%) after the first staging visit, whereas only 4.8% (4/83) were classified as Stage 2. The prevalence of Stage 2 is comparable to 7.7% (17/220) reported by the German

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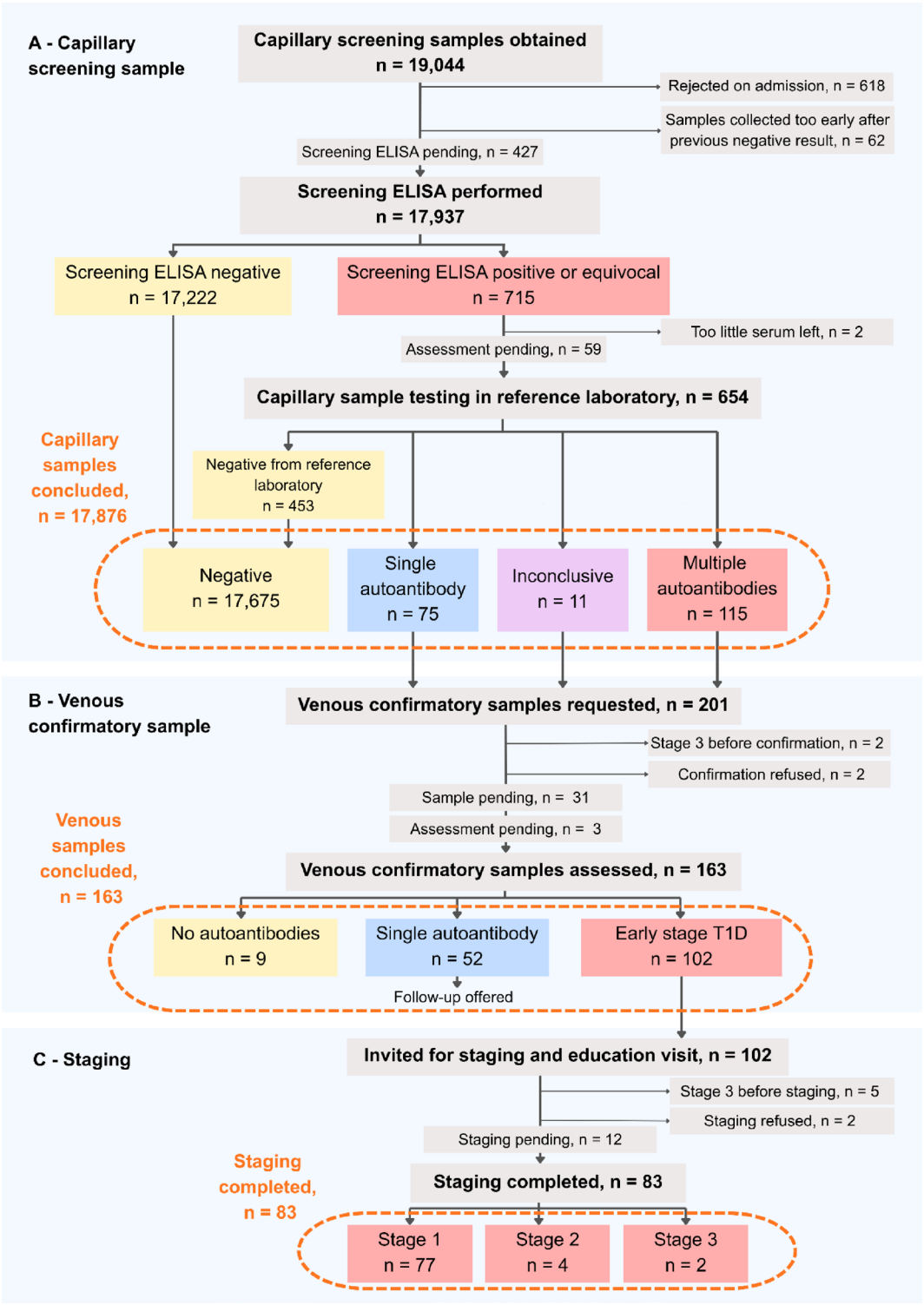


FIGURE 2 | Screening results and flow of samples.

programme Fr1da, and even the Australian Type1Screen with 15.4% (4/26) does not significantly differ from our proportion of subjects in Stage 2 ($p = 0.090$) [3, 13]. The seemingly infrequent Stage 2 among our children with early T1D may be connected with the diagnostic criteria used and will likely increase in the future. The EDENT1FI protocol adopted a conservative stance: to be classified as Stage 2, a child had to fulfil two criteria during a single visit or meet one criterion in two subsequent visits. The latter will likely lead to more children being reclassified into Stage 2 later during further follow-up visits, as one criterion will be fulfilled repeatedly. In contrast to us, Type1Screen classified children with multiple autoantibodies as Stage 2 even if only one criterion of dysglycaemia was present [13]. The Fr1da study also required only one dysglycaemic value to be met but did not use HbA1c as a criterion for Stage 2 [3]. Clearly, international comparability would benefit from unified reporting of the underlying tests, as is done under the EDENT1FI umbrella.

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TABLE 2 | Results from capillary screening samples.

Conclusion of the capillary screening	General population	Subjects having an FDR with T1D	Total subjects
n[a]	*n* = 16 027	*n* = 1849	*n* = 17 876
Concluded as negative	15 906 (99.2%)	1769 (95.7%)	17 675 (98.9%)
– Of negatives, conclusion made from the first-tier ELISA test	15 509 (96.8%)	1713 (92.6%)	17 222 (96.3%)
– Of negatives, conclusion made after the reference laboratory had refuted a suspect ELISA result	397 (2.48%)	56 (3.03%)	453 (2.53%)
Concluded as having two or more islet autoantibodies	63 (0.39%)	52 (2.8%)	115 (0.64%)
Concluded as having a single islet autoantibody	51 (0.32%)	24 (1.30%)	75 (0.42%)
Conclusion not reached—Inconclusive pattern; sample exhausted during the confirmation procedures[b]	7 (0.04%)	4 (0.22%)	11 (0.06%)

[a]For the 62 subjects with a repeated sample collected too early after the initial negativity, the first screening sample was counted.
[b]Capillary samples contained at least one autoantibody but it was exhausted during the confirmation procedure. These subjects were directed into the venous confirmation pipeline.

A notably high adherence to the follow-up protocol was noted among the families of children with newly identified early-stage T1D. Among 102 families of children diagnosed with early-stage T1D, only two (2.0%) refused to attend the staging and education visit. This is markedly less than the loss to follow up reported at this point from other screening programs: Type1Screen reported 27.8% lost (20/70 children), whereas Fr1da reported 15.7% (41/261) [3, 13]. A factor that might have supported the high adherence in our study is the direct contact established by the study team immediately after the diagnosis of early-stage T1D. The positive result was communicated to the family by a phone call from a trained physician of the project coordination site. During this call, the diagnosis and its consequences were explained to the family and they were invited for the staging and education visit. This visit was performed by the same physician. That ensured creating a personal bond with the family right from the beginning.

All education and staging visits were conducted at the study centre of the national coordinator. That ensured consistency in content on one hand but required long travelling for some of the families on the other. Regarding future perspective, more centres in Czechia will likely be conducting staging visits per standardised protocol. In other European screening programmes (Fr1da, ELSA), the staging visits are done close to the families' homes to offer more convenience [3, 29].

The project has generated valuable population-level data. Participants were evenly distributed across the paediatric age range (2.0–17.9 years) and throughout the whole country. Their samples were collected by 270 screening sites distributed across the whole country, which represents around 13% of the total of 2027 general paediatric practices operating in the country in 2024 [30]. To our knowledge, no previous T1D screening programme has achieved such nationwide coverage. We acknowledge that participation was voluntary and that the screened cohort is therefore not fully representative of the Czech general paediatric population. Nevertheless, the Betty project provides unique real-world data on population-based T1D screening from approximately 1% of the national paediatric population.

FDRs account only for 10.3% participants suggesting a high level of acceptance among the general population. However, compared to published data from other population-based screening projects, the number of FDRs in the Betty project is indeed higher. In the Fr1da study, FDRs accounted for 3.4% [3]. However, the target group of this screening was children aged 1.75–5.99 whereas we opted for a wider age range which allowed more FDR children to be tested. Results from American ASK screening study report 5% of FRDs among the screened children aged 2–17 [16]. Other population-based screening programmes like ELSA or ADIR have not published such data yet. Our rate of FDRs might be higher due to the possibility of home-testing which was more often used by FDRs. The share of self-collected samples was 19.5% among FDRs compared to 2.0% among the general population. Information about the launch of the Betty project spread quickly among parents of children with T1D and these families then actively sought the opportunity to participate in the screening even if they did not have a screening centre nearby. Their high interest in screening is understandable as they are more engaged in the topic than the general public. This also led to the high participation of FDRs in the initial months of the screening. After the initial interest levelled, the share of FDRs decreased

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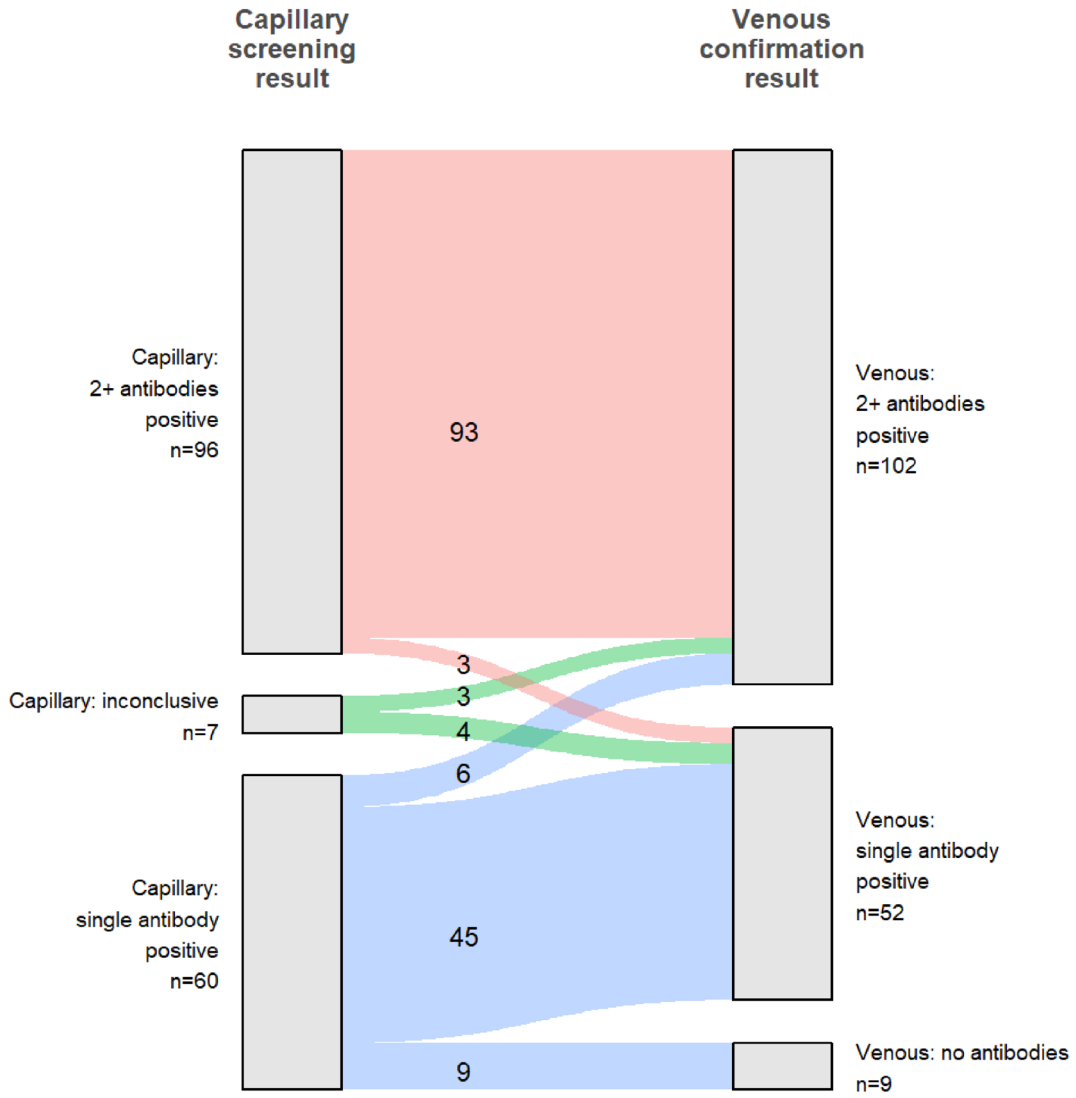


FIGURE 3 | The relation between the autoantibody results in non-negative capillary screening samples, and the subsequent venous confirmatory samples.

in time. To achieve a lower share of FDRs initially, we would need to restrain certain families from testing which was not our intention.

Having thus mapped the paediatric population, future implementation of a T1D screening within the Czech national healthcare system will likely narrow the focus on younger age groups to increase efficacy. Available evidence suggests that sensitivity and positive predictive value are highest when autoantibody screening is done at the age of 4 when tested once, and at ages 2 and 6 years when two consecutive screenings are done [31]. These screening intervals would naturally align with the regular biannual checkups done by primary care paediatricians. Such an alignment likely occurred naturally in the present study, as more samples were collected at odd ages when these visits occur in Czechia.

The positivity threshold for the first-tier ELISA assay clearly needs adjustment before the screening is introduced in routine clinical practice—primarily to relieve the burden of the secondary retesting in a reference laboratory. The EDENT1FI protocol is based on a two-tier autoantibody testing from the same capillary sample, and the threshold of the first-tier screening, the three-screen ELISA, was set rather low in order to decrease false negativity rates. Of the capillary screening samples received, 3.9% were sent for confirmation to the reference laboratory. Of them, only 25.9% were concluded to contain any islet autoantibody, with the positive predictive value highly dependent on the level of the compound signal from the first tier (Table S2). This exploratory post hoc analysis suggests that, if prospectively validated, increasing the first-tier screening ELISA threshold could help optimise the cost-effectiveness of the protocol for future implementation within the national healthcare system.

To our knowledge, none of the existing T1D screening programmes have reported real-world operational timelines, such as the time from blood collection to laboratory result and family notification, the time to venous confirmation, or the total time to diagnosis. Therefore, comparisons of our data with similar screening programmes worldwide are not currently possible. However, we believe that these operational metrics are highly relevant and should be routinely evaluated, as they provide

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valuable information on the feasibility and efficiency of large-scale screening programmes. According to recent consensus guidance by Phillip et al., the first positive test should be confirmed with a second test within 3 months [27].

We observed an approximately 2-month interval between collection of the capillary screening sample and communication of a positive result. Based on current knowledge of the natural course of T1D progression, such an interval is unlikely to substantially compromise the clinical benefit of early diagnosis [27, 32]. However, this interval remains important, primarily because of the psychological burden associated with waiting for the screening result. A significant reduction in the duration of the screening process would require implementation of LIPS and RBA testing locally in Czechia, which was not part of the current project but might be necessary if T1D screening were to be implemented into the national healthcare system.

Children with one autoantibody only were followed outside the EDENT1FI protocol. We requested a venous confirmatory sample, as data on significance of single-antibody positivity detected by screening are scarce. Previous prospective studies showed that titres of islet autoantibodies can fluctuate, and multiple autoantibody positivity may revert to single autoantibody positivity and vice versa [33]. Recent consensus guidance also recommends confirming single autoantibody positivity detected through screening with a second sample [27]. Of the 60 children with single autoantibody detected in the capillary screening sample, 6 (10%) seroconverted for an additional autoantibody already in the venous confirmatory sample taken a few months later (Figure 3 and Table S3). Here the second test sample has proven crucial for the decision about the clinical management.

The study has several limitations. Firstly, capillary screening samples in serum separator tubes are a robust collection system but are prone to bacterial decay and haemolysis during transport. Although only 3.2% samples had to be rejected on admission and no effect was noted of the haemolysis degree on the positivity proportion in screening ELISA, future protocol modifications may consider the use of dried blood spots. Secondly, the EDENT1FI protocol was designed for the screening of early-stage T1D and not to detect individuals with a single autoantibody positivity. Therefore, children who are positive for IAA only could be missed, as the screening ELISA test detects the summary signal of the other three autoantibodies (anti-GAD, anti-IA2 and anti-ZnT8). This cannot be presently remedied due to technical and financial constraints associated with accurate methods available for IAA testing.

Future evaluation of the programme will provide important insights into its population-level effectiveness, particularly regarding screening sensitivity and retention during long-term follow-up. As the screening programme has been available only since November 2023, longer follow-up is required to assess these outcomes.

In conclusion, the Betty project demonstrates that establishing a population-based screening programme based on the common EDENT1FI protocol is feasible and has laid a strong foundation for the potential implementation of population-based T1D screening within the national healthcare system.

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Ethics Statement

The study has been approved by the Ethics Committee of University Hospital Motol (Project No. 1271/23).

Conflicts of Interest

Z.S. and O.C. received consultations fees from Sanofi-Aventis. A.-G.Z. served as a member of advisory boards for Sanofi-Aventis and Novo Nordisk, and received support to give lectures sponsored by Sanofi-Aventis. C.M. serves or has served on the advisory panel for Novo Nordisk, Sanofi, Eli Lilly and Company, Novartis, Dexcom, Boehringer Ingelheim, Bayer, Roche, Abbott, Medtronic, Insulet, Biomea Fusion, SAB Bio, vIv Therapeutics and Vertex. Financial compensation for these activities has been received by KU Leuven; KU Leuven has received research support for CM from Dexcom, Novo Nordisk and Sanofi; C.M. serves or has served on the speakers bureau for Novo Nordisk, Sanofi, Eli Lilly and Company, Medtronic, Dexcom, Insulet, Abbott, Vertex and Boehringer Ingelheim. Financial compensation for these activities has been received by KU Leuven. C.M. is president of EUDF. All external support of EUDF is to be found on www.eudf.org. Other co-authors declare no conflicts of interest.

Data Availability Statement

The datasets generated during the current study are available from the corresponding author upon a reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Criteria for normoglycaemia, dysglycaemia and hyperglycaemia according to the EDENT1Follow Master protocol. **Table S2:** The threshold of the primary ELISA screening assay could be potentially raised without compromising sensitivity for detecting early-stage T1D. **Table S3:** Venous confirmatory samples and their breakdown by the pattern of autoantibodies found in capillary screening sample. **Figure S1:** The share of individual screening sites on the total of screened subjects. **Figure S2:** Distribution of capillary screening sample counts in time and by age. **Figure S3:** Positivity for the first ELISA test by the grade of capillary screening sample haemolysis. **Figure S4:** Time needed to obtain results and time between important check points. **Figure S5:** Time from a positive capillary result (two or more autoantibodies) to obtaining a venous confirmatory sample. **Figure S6:** A detailed breakdown of the state diagram by sample status. **Data S1:** List 'on behalf of EDENT1FI'.

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