BMJ Open

Universal screening for early detection of chronic autoimmune, metabolic and cardiovascular diseases in the general population using capillary blood (UNISCREEN): low-risk interventional, single-centre, pilot study protocol

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To cite: Merolla A, De Lorenzo R, Ferrannini G, *et al*. Universal screening for early detection of chronic autoimmune, metabolic and cardiovascular diseases in the general population using capillary blood (UNISCREEN): low-risk interventional, single-centre, pilot study protocol. *BMJ Open* 2024;**14**:e078983. doi:10.1136/bmjopen-2023-078983

► Prepublication history for this paper is available online. To view these files, please visit the journal online (https://doi.org/10.1136/bmjopen-2023-078983).

Received 17 August 2023
Accepted 29 January 2024

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ABSTRACT

Introduction Chronic autoimmune (type 1 diabetes and coeliac disease) and metabolic/cardiovascular (type 2 diabetes, dyslipidaemia, hypertension) diseases are highly prevalent across all age ranges representing a major public health burden. Universal screening for prediction/early identification of these conditions is a potential tool for reducing their impact on the general population. The aim of this study is to assess whether universal screening using capillary blood sampling is feasible at a population-based level.

Methods and analysis This is a low-risk interventional, single-centre, pilot study for a population-based screening programme denominated UNISCREEN. Participants are volunteers aged 1–100 who reside in the town of Cantalupo (Milan, Italy) undergoing: (1) interview collecting demographics, anthropometrics and medical history; (2) capillary blood collection for measurement of type 1 diabetes and coeliac disease-specific autoantibodies and immediate measurement of glucose, glycated haemoglobin and lipid panel by point-of-care devices; (3) venous blood sampling to confirm autoantibody-positivity; (4) blood pressure measurement; (5) fulfilment of a feasibility and acceptability questionnaire. The outcomes are the assessment of feasibility and acceptability of capillary blood screening, the prevalence of presymptomatic type 1 diabetes and undiagnosed coeliac disease, distribution of glucose categories, lipid panel and estimate of cardiovascular risk in the study population. With approximately 3000 inhabitants, the screened population is expected to encompass at least half of its size, approaching nearly 1500 individuals.

Ethics and dissemination This protocol and the informed consent forms have been reviewed and approved by the San Raffaele Hospital Ethics Committee (approval number: 131/INT/2022). Written informed consent is obtained from all study participants or their parents if aged <18. Results will be published in scientific journals and presented at meetings.

Conclusions If proven feasible and acceptable, this universal screening model would pave the way for larger-scale programmes, providing an opportunity for the implementation of innovative public health programmes in the general population.

Trial registration number NCT05841719.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The population-based design constitutes a valuable source for acquiring real-world data and extending the applicability of findings to a wider demographic context.
⇒ Capillary blood sampling provides a minimally invasive approach, particularly beneficial when considering the screening of children.
⇒ The point-of-care testing method provides immediate access to results for glucose and lipid profiles ensuring rapidity of screening procedure and real-time assessment of participants' health status.
⇒ The relatively small sample size may limit the generalisability of findings and necessitate cautious interpretation of the results.
⇒ The single-centre nature of the study may constrain the external validity of the results due to possible variations in health characteristics and demographics compared to different settings.

INTRODUCTION

Background and rationale

Screening, based on the use of easily applicable tests, is a public health strategy to identify individuals with unrecognised disease or disease risk factors before clinical onset. Prediction, prevention, early diagnosis and management are the fundamental goals of screening programmes within a defined population. Chronic autoimmune (including type 1 diabetes and coeliac disease), metabolic (including type 2 diabetes and dyslipidaemia) and cardiovascular (including hypertension)

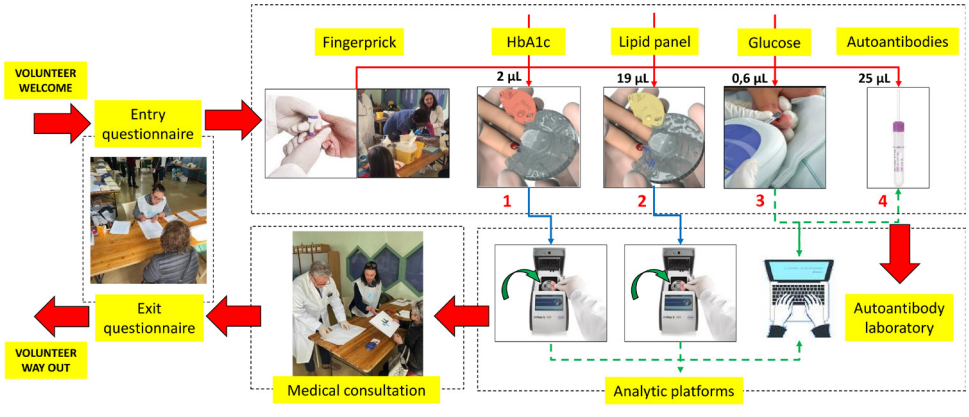
diseases are largely prevalent chronic health conditions across childhood, adolescence, adulthood and old ages, ideal targets for universal screening campaigns in the general population.

Type 1 diabetes is an autoimmune disease ensuing from the chronic destruction of insulin-producing β cells within the pancreatic islets of Langerhans.[1] Due to its continuously increasing incidence, especially in childhood and adolescent ages,[2] type 1 diabetes has become a major public health problem worldwide, requiring action at the local and global levels. Screening for the identification of subjects at risk is a fundamental step for the prevention of diabetic ketoacidosis (DKA), a severe, potentially lethal, complication of the disease associated with its late diagnosis and, in perspective, for the prevention of the disease itself, when new therapies able to modify the disease course will become available.[3] The autoimmune process underlying type 1 diabetes is identified by islet autoantibodies, detectable in the peripheral circulation years before clinical onset, including insulin (IAA), glutamic acid decarboxylase (GADA), tyrosine phosphatase IA-2 (IA-2A) and zinc transporter-8 (ZnT8A) autoantibodies. The screening for these serological markers allows the identification and staging of individuals at risk during their long presymptomatic period and accurately predicts their possible progression towards clinical manifestations of the disease.[4 5]

Coeliac disease is characterised by a chronic inflammation of the small intestine caused by an autoimmune mechanism triggered, in genetically predisposed individuals, by the ingestion of gluten with diet. Coeliac disease is relatively common in the general population, occurring at all ages, with a high proportion (even greater than 50%) of undiagnosed cases due to mild, or even absent, associated symptoms.[6] The incidence of coeliac disease is also estimated to be increasing, at least in children.[7] The most sensitive serological marker of coeliac disease, both for diagnosis and screening, are IgA-antibodies to tissue transglutaminase (TGA), requiring positivity for endomysial antibodies for diagnostic confirmation, while the need for additional duodenal biopsy in antibody-positive individuals, is debated.[8]

Type 2 diabetes, as well as any other form of diabetes, is diagnosed based on plasma glucose (fasting ≥126 mg/dL; 2-hour value during a 75 g oral glucose tolerance test (OGTT)≥200 mg/dL; or random ≥200 mg/dL) or glycated haemoglobin 1c (HbA1c) ≥6.5%. Intermediate categories between normality and diabetes include impaired fasting glucose (IFG) (fasting glucose from 100 mg/dL to 125 mg/dL) and impaired glucose tolerance (IGT) (2-hour glucose during OGTT from 140 mg/dL to 199 mg/dL).[9] Increased risk for future development of type 2 diabetes, also commonly referred to as prediabetes, includes any glucose abnormality of glucose, measured at fasting (ie, IFG),[10] post-meal (ie, IGT) and even random,[11] as well as A1c from 5.5% to 6.4%.[12] The same dysglycaemia conditions and elevated HbA1c are also predictive of cardiovascular (CV) diseases.[13–16] Prediabetes is frequently associated with obesity, dyslipidaemia and arterial hypertension, all representing their own independent CV risk factors.

Dyslipidaemia-associated CV risk is considered as part of a continuum, where lower levels of low density lipoprotein-cholesterol (LDL-C) are associated with lower CV risk. Nonetheless, ideal thresholds have been recommended at 116, 100, 70 and 55 mg/dL according to the total CV risk (low, moderate, high and very high) and it is of utmost importance for CV prevention to reduce LDL levels as soon as possible.[17] The goal in children >10 years of age is an LDL-C<3.5 mmol/L (<135 mg/dL) and at younger ages a ≥50% reduction of LDL-C.[18] With regard to this, familial hypercholesterolaemia (FH) deserves specific mention as an under-recognised and under-treated condition, contributing to a higher risk of premature heart disease. Paediatric FH screening, and subsequent guideline-based treatment, can dramatically improve young people's lives. A heart-healthy diet should be adopted early in life and statin treatment should be considered at 6–10 years of age. Several model programmes, both by measurement of cholesterol and



*Note: People depicted are not patients and photos were taken with the participant's knowledge.

Figure 1 UNISCREEN study procedures flow chart. *Note: People depicted are not patients and photos were taken with the participant's knowledge. HbA1c: glycated haemoglobin 1c.

genetic testing, have been experienced in Europe to identify young individuals with FH. However further research is needed to optimise care and implementations.[18]

Among CV risk factor management, arterial hypertension guidelines have also been recently revised,[19] indicating the value of <120 mm HG for systolic and <80 mm HG for diastolic office blood pressure (BP) as normal for the American and optimal for the European classification, respectively.

Accurate CV risk assessment can be estimated by combining an individual's characteristics with established risk factors (age, sex, glucose, systolic BP, cholesterol and smoking habit). Consequently, different specific lifetime CV risk scores should be considered and applied to both apparently healthy and already known CV disease-affected people, allowing the implementation of early tailored treatments and preventive strategies.[20] The main strategy of risk assessment employed to estimate the 10-year risk of fatal and non-fatal CV events (myocardial infarction, stroke) in apparently healthy people is the updated Systemic Coronary Risk Estimation (SCORE)2 algorithm, used to estimate the risk for individuals aged 40–69 years old[21] and SCORE2-Older Persons (OP) for individuals aged 70 years and older.[22]

Despite continuous progress and innovation in diagnostics and therapies over the last decades, the burden of chronic diseases in the general population is still high, especially in case of missed or delayed diagnosis. Making screening for their early identification accessible to the general population is a potential key to facilitating the public health mission of reducing the load of these conditions.

Blood tests are typically used for screening purposes; however, conventional venous blood sampling and laboratory methods may be relatively laborious and not well accepted due to venepuncture, especially in children. In contrast, simple, rapid and more tolerable capillary blood sampling by fingerprick could overcome these limitations and offer a valid alternative.[23] Moreover, in most cases, capillary and venous blood can be used for the same measurements, without affecting the validity of test results. Validated markers measurable in capillary blood include: islet autoantibodies for type 1 diabetes[24–26] and TGA autoantibodies for coeliac disease[27]; glucose[28] and HbA1c[29] for type 2 diabetes and dysglycaemia; total, high density lipoprotein (HDL)-cholesterol, LDL-cholesterol and triglycerides[30 31] for dyslipidaemia, including in children.[32]

Explanation for the choice of comparators

Not applicable, as the screening intervention is equally offered to the entire study population.

Objectives

The overall objective of this study is to perform a universal screening using capillary blood, in addition to anthropometric and BP measurement, for risk assessment for the main chronic autoimmune, metabolic and CV diseases in the general population.

We here report the study design, including feasibility and acceptability assessment, of a population study denominated UNISCREEN, conducted in Cantalupo, a municipality fraction in Northern Italy.

This study represents a model for a public health programme conducted in the general population across all ages, using capillary blood sampling for screening markers of the most prevalent chronic autoimmune, metabolic and CV diseases.

The primary outcome is to assess feasibility and acceptability. Additional outcomes are the identification of presymptomatic stages of type 1 diabetes, undiagnosed coeliac disease, type 2 diabetes, dysglycaemia and dyslipidaemia and risk assessment for CV diseases.

Trial design

This is a low-risk interventional, single-centre pilot study, carried out in a small town, assessing whether early staging of the most prevalent chronic autoimmune, metabolic and CV diseases in all age groups is feasible at a population-based level. Specific design features of this pilot project will be evaluated in terms of feasibility and acceptability to optimise the application to subsequent large-scale trials. Low-risk intervention refers to the following: (1) collection of capillary blood samples from fingerstick for autoantibody testing and point-of-care metabolic measures, with subsequent additional venous blood sampling in autoantibody-positive participants; (2) measurement of BP; (3) collection of demographic and anthropometric data and general health assessment conducted by a physician, with preliminary recommendations based on results of extemporary measurements; (4) administration to participants of questionnaires on feasibility and acceptability at the entry (pre-screening) and at the end (post-screening) of the procedure (figure 1). The study is planned to have a total duration of 10 months, with a screening campaign period of at least 6 months followed by an estimated additional period of 4 months for data analysis.

METHODS: PARTICIPANTS, INTERVENTIONS AND OUTCOMES

Study setting

This study is addressed to volunteers who reside in Cantalupo, a locality of 3061 inhabitants belonging to the Municipality of Cerro Maggiore (Province of Milan, Italy). The choice of this small locality follows an agreement between the study promoter and the Municipality of Cerro Maggiore, the Local Civil Protection and Fondazione Italiana Diabete (FID) with the role of contributing to screening programme conduction and accomplishment. The screening activity is carried out in Cantalupo main social gathering places, such as schools and public squares.

On 31 May 2022, the distribution of Cantalupo inhabitants was as follows:

- Number of inhabitants between 1 and 15 years of age: 404 (13.2% of the population).
- Number of inhabitants between 16 and 30 years of age: 425 (13.9%).
- Number of inhabitants between 31 and 45 years of age: 539 (17.6%).
- Number of inhabitants between 45 and 60 years of age: 729 (23.8%).
- Number of inhabitants between 61 and 75 years of age: 621 (20.3%).
- Number of inhabitants over 75 years of age: 343 (11.2%).

The study is intended to screen 50% or greater of the Cantalupo population. Therefore, enrolment of at least 1531 participants is expected to be realistic. The pilot study is intended to pave the way to future larger studies, including the recently approved Italian National Law for type 1 diabetes and coeliac disease screening in childhood and adolescence.[33]

Eligibility criteria

Participants are individuals aged between 1 and 100 years who live and have a residency in Cantalupo, enrolled voluntarily. They must have the ability to understand the purpose of the project and sign the informed consent. Consent of minors is signed by one of the parents. No exclusion criteria are defined for the present study. Interventions are performed in Cantalupo by a dedicated medical and nursing team from San Raffaele Hospital and local volunteering associations. Fasting is not requested for participation in screening.

Patient and public involvement statement

1. At what stage in the research process were participants first involved in the research and how?

Participants and public are actively involved in all the stages of the UNISCREEN research process. The study is funded by FID (a laypeople foundation for type 1 diabetes research, managed by people and families with type 1 diabetes) providing also non-medical staff volunteers and a well-operating network in connection with other non-governmental organisations, including the Local Civil Protection, Red Cross and some Rotary clubs, ultimately serving with more than 100 volunteers during the conduction of the screening. Before the initiation of the screening programme, the population of Cantalupo received adequate information about the opportunity to participate in the study through effective sensitisation and awareness campaigns. Volunteers from the Local Civil Protection, FID, Municipality Authorities and local primary care physicians and paediatricians all contributed by providing essential information and sensitisation. Additionally, traditional communication channels and door-to-door approaches were used to ensure widespread involvement and information dissemination.

2. How were the research question(s) and outcome measures developed and informed by their priorities, experience and preferences?

The proposed study was designed to address the urgent need for early detection or prevention of chronic diseases largely represented in the general population across ages (autoimmune, metabolic, CV). The delayed diagnosis implies an elevated burden on both patients and the healthcare system, possibly favouring the progression of chronic complications. The innovative study design of a universal large-scale screening, accessible to the general population, based on the simple capillary blood sampling by fingerprick conceived to overcome costs, time and organisational constraints of screening based on venous blood sampling was emphasised in respect to potential participants, in order to make them more compliant and gratified.

3. How were participants involved in the design of this study?

The workflow of the screening procedure has been planned by patients with diabetes from FID and Civil Protection volunteers, taking into consideration both the psychological and human issues related to participation and the operational and technical needs. Volunteers were consulted to assess the perceived burden of the intervention, time commitment for study participation, needs for a person undergoing a screening, special care required for kids and adolescent screening, the ideal sequence of point-of-care sampling and the overall design of the workflow, in order to improve the participant experience, reduce the timing of screening and any further possible burden. On the occasion of the screening procedure, each participant (or family) is accompanied by a non-medical volunteer through all the different phases of the screening, providing explanation and support, favouring confidence and increasing easiness. The feasibility and acceptability questionnaires have been reviewed by the patients of FID to ensure they were easily understandable to lay people, respectful of people with disease and easy to administer by non-medical volunteers to make participants free and as little conditioned as possible to answer.

4. How were they involved in the recruitment to and conduct of the study?

The choice of the city fraction of Cantalupo as the location for the screening programme comes from the promoter of the study FID and the collaborating Civil Protection. Thanks to them, an agreement with the municipality of Cerro Maggiore city was made, allowing the usage of the nursery school during weekends for screening activity, the involvement of the local schools, general practitioners, sports club, municipal association senior citizens centre, church and other relevant local entities. A general assembly has been organised to explain the study to the population and start the recruitment of participants, mainly done with the collaboration of the primary school, the senior citizens centre and the municipality itself.

5. Were they asked to assess the burden of the intervention and the time required to participate in the research?

More than 100 non-medical volunteers from FID, Local Civil Protection, Red Cross and Rotary clubs are involved during the operative on-the-ground phase of the screening: they organise screening appointments, set-up and secure the location, check the technical equipment, welcome and check participant IDs, explain the procedures of the screening, collect the informed consent and the privacy policy statement, collect the administrative information and demographics, administer the pre-screening feasibility and acceptability questionnaire, follow the participant during the capillary blood sampling, measure the BP (Red Cross volunteers), follow and check the correct operation of the point of care machines, collect the results, enter the data, bring participants to the medical consultant and finally administer the post-screening feasibility and acceptability questionnaire. All volunteers are trained during two meetings of 2 hours each, receive a handbook on the study and procedures, are involved in on-the-ground tests and are followed by senior volunteers.

6. How were (or will) they be involved in your plans to disseminate the study results to participants and relevant wider patient communities?

Once published, all study results will be presented, explained and discussed together with the population during public debates and specifically organised meetings in the schools and other public places, where researchers and volunteers will be available to reply to any possible question. Results will also be disseminated on the media (local and national) and a special open access live with Q&A on the social media of FID will be held.

Intervention description

The screening campaign opened on 22 April 2023. Before the starting date, Cantalupo population has been adequately informed about the possibility of participating in the study. Volunteers from the Local Civil Protection, FID, as well as Municipality Authorities and local primary care physicians and paediatricians contributed to the community information and sensitisation. Usual communication channels and door-to-door approaches have also been used.

The screening procedures take place in defined meeting points in Cantalupo, social gathering places, and public buildings such as schools or squares. A dedicated team of medical and nurse professionals from the San Raffaele University Hospital and local volunteer associations are responsible for carrying out the screening procedures.

Participants are enrolled voluntarily, on informed consent acquisition. During the screening visit, each participant undergo the following procedures (figure 1): (1) identification, collection of signed informed consent and privacy policy statement, administrative information and demographics; (2) measurement of BP by sphygmomanometer, according to standardised validated technique; (3) one or more fingerpricks for capillary blood drawing, used for collection in microtubes (Microvette 200 Z tubes) of at least 25 µL for future autoantibody assays and for extemporary measurements of glucose (0.6 µL) by glucometer and HbA1c (6 µL) and lipids (total, HDL-cholesterol and LDL-cholesterol and triglycerides) (19 µL) by point-of-care devices; sampling of venous blood is obtained in case of capillary blood drawing failure; (4) physician interview on anthropometrics (reported weight and height) and medical history (with specific focus on diabetes, coeliac disease, CV events and current therapies) using specific questionnaires, followed by communication, discussion and possible preliminary recommendations based on results of extemporary metabolic and BP measurements; (5) fulfilment at the entry (pre-screening) and end (post-screening) of a structured feasibility and acceptability questionnaire to evaluate participants' understanding about the instruction notice and their opinion or level of satisfaction with the practicability of the capillary test. Fasting is not required and post-prandial time is recorded.

Blood capillary samples for autoantibody measurements are stored in a refrigerator at 3–5°C and brought to the laboratory for up to 48 hours. On receipt, they are centrifuged and serum samples are stored at −20°C until testing. GADA, IAA, IA-2A, ZnT8A and TGA are measured by luciferase immunoprecipitation system (LIPS) (or LIPS converted from radio-binding assay - RBA) assays as previously reported.[34–38] Extemporary measurements using capillary blood by point-of-care devices include glucose (0.6 µL, Accu-Chek Inform II),[28] HbA1c (2 µL, Cobas b 101 Roche)[29] and lipid panel, including total cholesterol and HDL-cholesterol,[30] triglycerides[31] and LDL-calculated cholesterol (19 µL, Cobas b 101 Roche).[29] Results of extemporary measurements are available in a few minutes, reported in the patient case report form (CRF), communicated and discussed by the physician in the interview with the participant. Participants who are lately informed about the positivity of autoantibody screening either for type 1 diabetes or coeliac disease on capillary blood, are invited to undergo an additional venous blood sampling for confirmation.

Criteria for discontinuing or modifying allocated interventions

Expression by a participant of unwillingness to continue once the procedure has started, for any reason. They may also decide not to undergo the additional sampling for autoantibody confirmation for any reason.

Strategies to improve adherence to interventions

At the screening site, a pool of dedicated physicians, nurses and volunteers take care of any single participant or family, accompany them throughout the procedure and help them in case of any needed assistance.

Relevant concomitant care permitted or prohibited during the trial

Not applicable, since no concomitant care restrictions are planned for the study protocol.

Outcomes

Primary

Assessment of feasibility and acceptability of a capillary blood screening conducted in the general population across all ages for the main prevalent chronic autoimmune, metabolic and CV diseases.

A mixed qualitative and quantitative methods approach will be adopted to establish feasibility and acceptability, as outlined below: recruitment and retention rates; time required to recruit to target; rate of completion of the intervention (ie, number of participants who access and complete all aspects of the project); feasibility of testing procedures and data collection; patients' views and experiences of the intervention, caregiver reaction to having their child screened, overall impressions on the safety of the protocol.

If the intervention is found to be both feasible to deliver and acceptable by participants, we will establish whether to proceed with a larger sample size, following minor modifications if required. Outcome data for at least 50% of the population will be required to support our decision on whether the model is successful and applicable on a larger-scale basis.

Secondary

Determine the prevalence of presymptomatic type 1 diabetes by screening for IAA, GADA, IA2A and ZnT8A and the prevalence of asymptomatic/latent coeliac disease by screening for TGA. Cut-off for autoantibody positivity will be initially based on standard references, then recalculated by receiver operating characteristic curves using the entire cohort investigated. Screening samples positive for autoantibodies will require confirmation from a second venous blood sample with repeated measurements of all autoantibodies, with the addition of endomysial antibodies in TGA-positive participants. Further staging of presymptomatic type 1 diabetes[5] and diagnosis of coeliac[8] will be then established.

Evaluate the prevalence of diabetes based on random glucose ≥200 mg/dL and/or A1c≥6.5% criteria. Random glucose <200 mg/dL will be non-diagnostic, while HbA1c of 5.7–6.4% will be suggestive of pre-diabetes.[9]

Assess the distribution of total, HDL-cholesterol and calculated LDL-cholesterol and triglycerides, both in adults and children, and categorise it according to the different thresholds for low, moderate, high and very-high risk.[17 39–43]

Determine the prevalence of arterial hypertension in adults (systolic BP≥120 mm Hg, diastolic BP≥80 mm Hg)[19] and in paediatric (systolic BP≥120 mm Hg, diastolic BP≥80 mm Hg) population.[44]

Estimate 10-year CV disease risk using SCORE2 and SCORE2-OP algorithms.[21 22]

Participant timeline

The study was started in April 2023 and has an expected duration of 10 months. For the next few months, screening activities will be held every week-end at defined meeting points in Cantalupo (schools, public squares). Each participant is enrolled after obtaining the informed consent. During the screening visit, participants complete the questionnaires and undergo capillary blood draw. One sample is immediately analysed for glucose, HbA1c and lipids measurement by point-of-care devices; the remainder one is analysed later at San Raffaele Hospital Laboratory for autoantibodies measurement. Autoimmunity results will be communicated to the participant when available. If autoantibody screening is positive, participants will be invited for a confirmatory venous sample.

Sample size

The town of Cantalupo has a total of 3061 inhabitants. The study sample is expected to represent at least 50% of the population (N=1531).

Recruitment

To achieve adequate participant enrolment, intensive and well-organised community sensitisation strategies are needed. Also, usual communication channels and door-to-door approaches are needed to maximise the information spreading about study procedures. Volunteers from the Civil Protection, FID, parish church, local primary care physicians and paediatricians can contribute to the fulfilment of the project.

METHODS: ASSIGNMENT OF INTERVENTIONS

Allocation

Sequence generation

Each participant is assigned a specific computer-generated alphanumeric identification code. Data collected from the study are registered and linked to this code. Therefore, all the study information is stored anonymously. Only study physicians and authorised staff can link the code to the participant's name.

Concealment mechanism

Not applicable, it is not a controlled trial, and there are no different study groups.

Implementation

Physicians are in charge of enrolling participants who meet all the eligibility criteria. Each participant is assigned an alphanumeric identification code from a computer-generated list produced by San Raffaele Hospital statisticians.

Blinding

Who will be blinded

Not applicable. Study participants, screening procedures and data analysts will not be blinded.

Procedure for unblinding if needed

Not applicable, there is no blinding.

METHODS: DATA COLLECTION, MANAGEMENT AND ANALYSIS

Plans for assessment and collection of outcomes

Data collected from questionnaires and laboratory tests are registered in a CRF, and then stored in the principal investigator's office (Professor Emanuele Bosi). The source documentation reporting the patient's data is anonymised, using the predefined study code for each patient.

Plans to promote participant retention and complete follow-up

There is no specific plan for promoting participant retention and complete follow-up. The participation in the screening campaign is on a voluntary basis.

Data management

After the monitoring activity, data are entered into an Excel file, with restricted access (required password) stored on the institutional computer of the data manager and placed in his study. When planned in the protocol, the anonymised data will be transferred to the statistician for the statistical analysis.

Statistical methods

Statistical methods for primary and secondary outcomes

Continuous variables will be described by mean and SD or median and IQRs as appropriate depending on the final distribution. Categorical variables will be described by numbers and proportions of subjects in each category. X^2 test or Fisher exact test will be used for inter-groups comparison of categorical variables. Student's t-test will be used for inter-groups comparison of continuous variables with normal distribution. Non-parametric Wilcoxon rank-sum test will be needed for inter-groups comparison of continuous variables without normal distribution. Linear correlations between parametric continuous variables will be tested by Pearson correlation coefficient, while Spearman correlation coefficient will be used for non-parametric variables. Logistic regression models will be applied for predictive analytics of binary outcomes; ORs and related 95% CIs will be reported. Statistical analyses will be performed using R statistical package (V.4 or later), or other dedicated software, with a two-sided significance level set at $p<0.05$. When data analysis is completed, findings will be shared and proposed for publication in international scientific journals and/or they will be shared anonymously during professional activities at San Raffaele Hospital. The feasibility and acceptability of the study will be assessed as described above. The study will be considered successful if at least 50% of the population will be enrolled.

METHODS FOR ADDITIONAL ANALYSES (EG, SUBGROUP ANALYSES)

Estimation of the prevalence of the screened diseases across age classes is included among secondary outcomes.

METHODS IN ANALYSIS TO HANDLE PROTOCOL NON-ADHERENCE AND ANY STATISTICAL METHODS TO HANDLE MISSING DATA

The primary outcome analysis will include all participants. A low drop-out rate is expected; therefore, missing data will not be replaced, and data analysis will be performed based on the observed cases principle.

METHODS: MONITORING

Composition of the data monitoring committee, its role and reporting structure

Study endpoints are clear and the study duration is supposed to be brief. A comprehensive quality control will be set in the form of monitoring and will include checking the whole course of the study, management of documentation and data, management of enrolled patients and biological samples. The promoter of the study is responsible for monitoring. The investigators and researchers involved in the study should ensure the monitoring of the clinical study to guarantee conformance to protocol as well as the completeness, correctness and plausibility of the completed CRF. The study is conducted in accordance with the approved protocol, Good Clinical Practice and standard procedures. Promoter has to inform San Raffaele Hospital Clinical Trial Centre of any scheduled inspection by the Ethics Committee and provide a copy of any inspection report received.

Interim analyses

No interim analysis is planned.

Adverse event reporting and harms

No substantial study-related adverse events are expected. BP measurement and capillary blood sampling are rather easy and safe procedures. Some potential adverse events could be those related to pain and bruising at the site of blood sampling, allergic reactions to disinfectant solutions and/or gloves and/or adhesive patches, or even vasovagal reactions triggered by the sight of needles or blood. Additionally, the prospect of psychological impact, such as anxiety, or even depression, deriving from the communication of a possible previously unknown diagnosis, has to be taken into account. With regard to the results immediately available, such as metabolites and BP, the psychological distress may be curbed by the medical consultation at the end of the screening itinerary, where all the medical implications and therapeutic suggestions can be discussed directly with participants and their families (see section Intervention description). Differently, the late communication of a possible positivity of type 1 diabetes and coeliac-specific autoantibodies, might be distressful and deserves specific attention: an increased anxiety in mothers of children at the time of communication of presymptomatic type 1 diabetes has been reported, declining during follow-up.[45] Although there are no specific guidelines or recommendations, in UNISCREEN all the positive autoantibody results are personally communicated on the phone by the principal investigator, together with the recommendation of confirmatory tests on a venous blood test (see section Intervention description). In case of confirmed positivity for type 1 diabetes-associated autoantibodies, participants

will be offered to be followed within EDENT1FI, a newly established European consortium on preclinical type 1 diabetes, with specific protocols for the psychological impact of screening and clinical follow-up under development.

Frequency and plans for auditing trial conduct

A comprehensive quality of the study will include checking the whole course of the study, the documentation, statistical analyses and the investigators, if necessary. The promoter guarantees the availability for the inspections from the regulatory agencies.

Ethics and dissemination

Research ethics approval

This protocol and the template informed consent forms have been reviewed and approved by San Raffaele Hospital Ethics Committee: approval number: no. 131/INT/2022. Written informed consent to participate will be obtained from all study participants. The study has been registered at ClinicalTrials.gov.

Plans for communicating important protocol amendments to relevant parties (eg, trial participants, ethics committees)

Any changes to the protocol that may affect the conduct of the study or patient's safety, including changes in study objectives, design, sample size, procedures or significant administrative aspects, require a formal amendment to the protocol. It will occur on Ethics Committee approval, under the request of the principal investigator. Any occurrence that does not comply with the protocol, including protocol deviations, will be documented and reported by the principal investigator to the San Raffaele Clinical Trial Centre and the Ethics Committee. Administrative changes, minor corrections and/or clarifications of the protocol that do not affect the study conduct will be notified to the Ethics Committee. All modifications will be tracked and specified also in the final report. Trial participants will be informed of any substantial modifications.

Who will take informed consent?

Every participant is informed about the modalities of the clinical study in accordance with the participant informed consent document, both in writing and verbally, by the investigating physician. The informing physician and the participant must each personally sign and date the informed consent form with a declaration on data privacy. For minor participants (younger than 18 years), the informed consent is signed by one of the parents. Every informed consent is stored as part of the investigator's file and a copy must be retained by each participant.

Additional consent provisions for collection and use of participant data and biological specimens

No additional consent provisions are planned.

Confidentiality

The study has been approved by the San Raffaele Hospital Ethics Committee. Prior to enrolment, all participants are adequately informed regarding all aspects concerning the study, using absolutely clear language and terms. Consequently, they sign a dedicated informed consent document, while consent for participants under 18 years of age is signed by one or both parents or by the legal guardian when needed. Participation in the study is on a voluntary basis. The study is conducted in accordance with Declaration of Helsinki, observational/low-risk intervention study rules and Good Clinical Practice principles, in order to ensure the safety of study participants and the validity of the data. Moreover, in accordance with European Regulation 2016/679 and the current Italian Data Protection of Personal Authority, the collection and storage of personal health data is limited to study-related information and cannot be disclosed, made available or otherwise used. All laboratory samples, reports, data collection and administrative forms are identified by a coded ID number to maintain participant confidentiality. Only the physician and authorised personnel may be able to link this code to the participant identity. All the information is stored in locked files with limited access. All local databases are secured with password-protected access systems. Participant study information cannot be released outside of the study without the written permission of the participant, except as necessary for monitoring by the Ethics Committee, Italian and Foreign Regulatory Authorities.

Competing interests

The authors declare that they have no competing interests.

Access to data

The trial sponsor/principal investigator will have access to the final trial data set. There are no contractual agreements that limit such access. Any pertinent information is available from the sponsor/principal investigator upon reasonable request.

Provisions for ancillary and post-trial care

Paediatricians and primary care physicians are accurately informed of their patients' screening results with a letter including a brief summary of the purpose of the study and a copy of the document containing their patients' screening data. The letter may also incorporate the physician's suggestions regarding lifestyle interventions that participants should follow based on their cardiometabolic assessment. In case abnormal lipid and/or glucose levels are detected, the physician may recommend the paediatrician or the primary care physician run a confirmatory venous blood test to avoid any possible uncertainty.

This is to enable prompt preventive and/or therapeutic intervention as needed in order to guarantee continuity of care. In addition, at the time of screening, in case participants are found to have blood glucose or BP levels with potentially serious short-term consequences (eg, hypertensive crisis or hypoglycaemia), a dedicated nursing and

medical team are on hand to ensure the safety of participants in accordance with Good Clinical Practice.

Dissemination plans

Screening test resulting from rapid-reading tests are immediately shared with the participants. Results of lately performed autoantibody measurements will be available within 6 weeks and will then be transmitted to participants via phone contact or email. In case of positivity for either type 1 diabetes or coeliac disease-specific autoantibodies, participants will be personally informed by the principal investigator or some other staff physicians and subsequently invited to undergo a venous blood drawing for confirmatory purposes. All the screening results are also shared with the local primary care physicians and paediatricians. Collected data and study results will be the property of IRCCS San Raffaele Hospital, being the study promoter. The promoter is responsible for preparing a final report on the clinical study. There is no dissemination plan other than the publication of results in scientific journals and meetings, seminars or conferences, when appropriate.

Authorship eligibility

All authors have contributed substantially to the study in terms of conception or design, data acquisition, analysis or interpretation, intellectual content development or critical review and approved the article in its final version. In detail: Emanuele Bosi conceived, designed and oversights the study, participated in person-to-screening procedures, is responsible for data analysis, wrote the protocol and the paper; Aurora Merolla participates in person to screening procedures and wrote the paper; Rebecca De Lorenzo wrote the protocol; Cristina Renzi conceived the feasibility and acceptability part of the study; Giulia Ferrannini critically reviewed the protocol and contributed to the metabolic component of the study; Francesca Ulivi conceived and supervised the study organisation, participates in person to screening procedures and wrote the patient and public involvement statement paragraph; Elena Bazzigaluppi is responsible for autoantibody measurements; Vito Lampasona developed the LIPS technology for autoantibody assays, is responsible for autoantibody measurements, analyses and interpretation. There is no contribution by professional writers.

Plans for granting public access to the full protocol, participant level-data and statistical code

After the end of the study, full protocol and data will be available on request to the principal investigator.

Informed consent

Copies of informed consent for participants are available as attached documents.

Plans for collection, laboratory evaluation and storage of biological specimens for genetic or molecular analysis in this trial and for future use in ancillary studies (33).

No specimens for genetic analyses are collected. Any future research projects will be submitted for approval to the Ethics Committee of San Raffaele Hospital.

DISCUSSION

This study addresses the current and urgent need to handle the growing burden of the most prevalent chronic autoimmune, metabolic and CV diseases in the general population, by proposing a universal screening procedure based on capillary blood sampling, in addition to anthropometric and BP measurements. The overall objective is to improve public health outcomes through better prediction, prevention, early diagnosis and management across all age ranges.

This study complies with the general principles of screening, particularly: the conditions screened for represent a considerable burden within the general population; their natural history is known, with recognisable presymptomatic phase; screening measures hold acceptable sensitivity and specificity; early diagnosis allows prompt treatments, improve prognosis and prevent complications.[46] Moreover, feasibility and acceptability are included as the primary outcome of the study.

To our knowledge, this is the first study introducing blood capillary screening as a procedure to be used universally, across all age groups.

The chronic diseases investigated in this study include autoimmune type 1 diabetes and coeliac disease and the metabolic/CV diseases, or risk factors, type 2 diabetes, dysglycaemia and dyslipidaemia, all with validated markers measurable in capillary blood.[24–32]

Feasibility and acceptability have already been shown in type 1 diabetes screening using capillary blood sampling,[26] with advantages over outpatient venipuncture, particularly in children.[47]

Islet autoantibody screening proved to be accurate in identifying presymptomatic stages of type 1 diabetes[45] and effective in reducing the frequency of the associated DKA at the time of clinical onset.[48] Likewise, TGA antibody screening is effective in identifying both symptomatic and silent or asymptomatic coeliac disease.[8]

Point-of-care testing for glucose, HbA1c, cholesterol and related lipids provides immediate information for risk assessment of diabetes, glucose and lipid abnormalities and CV diseases and validated devices are now increasingly used in clinical practice to optimise patient care.[49] Remarkably, capillary screening for lipid abnormalities has recently been proposed and validated also in children, for the measurement of LDL-hypercholesterolaemia.[32 50]

In this study, for practical reasons, capillary blood is obtained in non-fasting conditions, as in most large population studies. Fasting is not a necessary prerequisite for the measurement of autoantibodies, HbA1c,[51] total and HDL-cholesterol,[52] while non-fasting testing only marginally changes triglyceride and therefore calculated LDL-cholesterol.[53 54] Conversely, non-fasting triglycerides have emerged as an increasingly important

risk factor[55] and non-fasting sampling for lipids is recommended by current guidelines for general screening and CV risk assessment.[20 56] Non-fasting, that is, random, glucose is also a useful category in the diagnosis of diabetes[9] and may be also predictive of future type 1 diabetes.[57]

In summary, the proposed screening study has the strength of simultaneously assessing the risk for the most prevalent chronic autoimmune, metabolic and CV diseases across all ages using the easily accessible, non-fasting, capillary blood sampling. If proven feasible and acceptable, this universal screening model would pave the way for larger-scale programmes, providing an unprecedented opportunity for the implementation of innovative public health programmes in the general population.

Trial status

Protocol V.1.0 7 November 2022. Recruitment began on 22 April 2023 and ended on 16 December 2023.

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Acknowledgements Authors are indebted with all physicians, nurses, technicians and lay volunteers for their willingness to contribute to the study.

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Funding The UNISCREEN Study was funded in part by a donation from Fondazione Italiana Diabete (FID), Milan, Italy (FID grant 07/2023).

Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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