Blood-free risk scores and neuropathy assessment tools to detect undiagnosed type 2 diabetes in Peru.

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Declaration of Authorship

I, Antonio Bernabe Ortiz, confirm that the work presented in this thesis in my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed: ____________________ Date: ___________________

Use of published work

Two papers have been published based on the work conducted for this thesis (Appendices A and B). Research for these papers was carried out as part of the PhD and was done during the period of registration of the PhD. The papers were part of the Chapter II and III of this thesis and include passages, tables and figures used. For both papers, Antonio Bernabe Ortiz (ABO) was the lead and the corresponding author, and was responsible for the literature review, analyses, and preparation of the first version of those papers. The co-authors' contributions to these manuscripts were restricted to providing comments on the draft prepared by ABO.

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Dedication

To my loving wife Katherine and my beloved son Gabriel, the reasons of my life; this work would have not been possible without them.

Abstract

The prevalence of type 2 diabetes mellitus is rising, especially in low- and middle-income countries, where the situation is worsened because around half of cases are unaware of the disease. Universal screening utilizing blood markers can be challenging in resource-constrained settings. The identification of these individuals can be potentially addressed using risk scores and neuropathy assessment tools. This study aimed to assess the diagnostic accuracy of the FINDRISC, a blood-free risk score, three neuropathy assessment tools (EZSCAN, pupillometer, and biothesiometer), alone and in combination.

A population-based study was conducted enrolling a sex-stratified random sample of participants from Tumbes, a semiurban area in the north of Peru. Undiagnosed T2DM was the outcome, defined using WHO OGTT thresholds. Diagnostic accuracy of the FINDRISC and neuropathy tools was evaluated using the area under the ROC curve (aROC) and respective 95% confidence intervals (95%CI).

Data from 1609 participants were analysed, mean age 48.2 (SD: 10.6) years, 810 (50.3%) females. A total of 176 (10.9%) individuals had T2DM, and only 71 (4.7%) had undiagnosed T2DM. The diagnostic accuracy of the FINDRISC was aROC = 0.69 (95% CI: 0.64–0.74), with a sensitivity of 69% and specificity of 67%. Among devices, the EZSCAN (aROC = 0.59; 95%CI: 0.53–0.66; sensitivity of 59% and specificity of 54%) and biothesiometer in the third metatarsal head (aROC = 0.60; 95%CI: 0.53–0.67; sensitivity of 31% and specificity of 85%) performed best. A combination of the FINDRISC and the biothesiometer had the best diagnostic accuracy, with a similar aROC of FINDRISC alone (AROC = 0.69; 95%CI: 0.68–0.78), with a sensitivity of 79% and a specificity of 59%.

Our results confirm that combination of the FINDRISC and biothesiometer can improve diagnostic accuracy of the FINDRISC and biothesiometer alone, increasing sensitivity without affecting specificity or the area under the ROC curve.

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List of Abbreviations

95% CI:	95% Confidence Intervals
AUC:	Area under the ROC curve (aROC)
BMI:	Body Mass Index
CINAHL:	Cumulative Index of Nursing and Allied Health Literature
DOR:	Diagnostic Odds Ratio
DTA:	Diagnostic Test Accuracy
ENINBSC:	National Survey of Nutritional and Biochemical Indicators for Non-communicable Diseases
FG:	Fasting Glucose
FINDRISC:	Finnish Diabetes Risk Score
GOD:	Glucose Oxidase
HbA1c:	Glycated haemoglobin
HDL:	High-Density Lipoprotein
HMIC:	Health Management Information Consortium
HS-ROC:	Hierarchical Summary Receiver Operating Characteristic
IDF:	International Diabetes Federation
IGM:	Impaired Glucose Metabolism
IPAQ:	International Physical Activity Questionnaire
LMIC:	Low- and middle-income countries
LR+:	Positive likelihood ratio
LR-:	Negative likelihood ratio
NICE:	National Institute for Health and Clinical Excellence
NVP:	Negative predictive value
ODK:	Open Data Kit
OGTT:	Oral glucose tolerance test
OR:	Odds ratio
PEN:	Peruvian Nuevos Soles
PPV:	Positive predictive value

PRISMA:	Preferred Reporting Items for Systematic Review and Meta-Analysis
QUADAS-2:	Quality Assessment of Diagnostic Accuracy Studies (Version 2)
ROC:	Receiver Operating Characteristic
SD:	Standard deviation
STARD:	Standards for Reporting Diagnostic Accuracy Studies
T2DM:	Type 2 diabetes mellitus
TRIPOD:	Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis
WHO STEP:	World Health Organization Stepwise approach to Surveillance

Chapter I: Introduction

1.1 Burden of T2DM in low and middle income countries

Globally, there is an increase in the burden of type 2 diabetes mellitus (T2DM): the age-standardised prevalence of T2DM has increased from 4.3% to 9.0% among men, and from 5.0% to 7.9% among women in the last four decades [1]. Moreover, T2DM is responsible for about 2 million deaths every year worldwide [2, 3], it is one of the leading causes of disability [4], and between USD$ 727 and 825 billion are estimated to be spent in T2DM-related healthcare [1, 5].

The burden of T2DM has increased faster during the last years in low- and middle-income countries (LMIC) compared to high-income countries [1]. In addition, T2DM imposes an extra risk among individuals from resource-constrained settings as, on average, 50% (range: 38% to 69%) of subjects with T2DM are not aware of their diagnosis [5]. Individuals with undiagnosed T2DM are usually asymptomatic until further complications, at the micro- and macro-vascular level, are clinically evident [6]; nevertheless, the scarcity of economic, human and infrastructure resources in resource-constrained setting might reduce the identification of T2DM cases.

1.2 Diagnosis of T2DM

Oral glucose tolerance test (OGTT) is considered one of the gold standards for T2DM diagnosis according to international guidelines [5, 7]. Despite of this, conventionally, fasting glucose is used in most of healthcare facilities in the world. OGTT and FG require at least eight hours of fasting; and in addition, OGTT also needs the patient to drink a 75-gram glucose solution and two blood samples, one at the beginning of the test, and then wait two hours before drawing the second blood sample [7]. Based on international standards, individuals who are not aware of having T2DM diagnosis and have fasting glucose level ≥126 mg/dL (≥7.0 mmol/L) or 2-hour plasma glucose ≥200 mg/dL (≥11.1 mmol/L) are classified as having screen-detected T2DM or newly-diagnosed T2DM. For our purposes, this definition is compatible with the definition of undiagnosed T2DM.

In 2009, the American Diabetes Association suggested that glycated haemoglobin (HbA1c) could be used as a diagnostic tool for T2DM [8] and is included in the current guidelines [7, 9]. HbA1c can give an idea of individual's plasma glucose levels over the previous 8 to 12 weeks [10], and assessment does not require fasting, but it can be expensive, especially in resource-constrained settings. In addition, HbA1c results need to be traceable to the Diabetes Control and Complications Trial reference study as certified by National Glycohemoglobin Standardization Program [11], which requires high-quality laboratory processes and standards. Moreover, a relatively recent pooled analysis found that HbA1c is not sensitive enough to detect cases of undiagnosed T2DM when compared to FG at the population level [12]. This latter pooled analysis also showed that HbA1c sensitivity varied across different world regions perhaps because of discrepancies between glycated haemoglobin and glycaemia in different racial and ethnic groups [13], as well as setting characteristics. In that sense, the relationship between glucose levels and HbA1c seems to be no evident at high-altitude areas compared to settings at the sea level [14]. Thus, the relationship between HbA1c and fasting plasma glucose looks quadratic at the sea level, whereas it was linear at high altitude, which turns in less reliable estimates. For instance, using recommended HbA1c cut-offs for T2DM screening would translate in major discrepancies in diagnostic performance (i.e. reduction of sensitivity from 89% at the sea level to 41% at high altitude settings) [14].

1.3 The Peruvian context

There are relatively scarce population-based studies estimating the prevalence and incidence of T2DM in Peru. Two national representative surveys, conducted at least five years apart, have estimated the prevalence of T2DM in Peru using fasting glucose to define cases of type 2 diabetes mellitus. The National Survey of Nutritional and Biochemical Indicators for Non-communicable Diseases (ENINBSC in Spanish), carried out between August 2004 and April 2005, found a T2DM prevalence of 5% [15]; whereas a most recent report, the PERUDIAB Study, conducted between 2010 and 2012, but including only Peruvian urban areas, found a prevalence of 7% [16]. Nevertheless, a relatively recent report also show that T2DM prevalence, defined using fasting glucose, differed according to study setting characteristics; thus, prevalence of T2DM varied from 3.1% in rural high-altitude areas to 10.3% in semiurban coastal settings [17]. Thus, no previous study has estimated the prevalence of T2DM in Peru using OGTT.

According to the Global Burden of Disease, T2DM prevalence in Peru is in the average (8.8%) [18]; however, the proposed area has rates over 10% [17]. Thus, the prevalence of the proposed setting is comparable to that in the Middle East and North Africa and mainly the North America (Mexico) and Caribbean region. In addition, prevalence of overweight and obesity is on average similar to other Andean Latin American countries (i.e. Bolivia and Ecuador) as well as Central Latin America (i.e. Colombia, Panama and Mexico). As all of these countries have similar socioeconomic profile (i.e. emerging economies), results of the proposed study may be useful for these similar areas.

Levels of awareness, treatment and control are also worrying. Using data of the PERU MIGRANT Study [19], overall T2DM diagnosis awareness was 71%, yet estimates ranged from 0% in rural settings to 74% in urban areas [20]. Among those aware of diagnosis, only 40.6% were on treatment, and among those in treatment, none was appropriately controlled using the HbA1c criteria (<7%). On the other hand, results using the baseline of the CRONICAS Cohort Study [17] showed that, among all T2DM cases, 61.3% were aware of their diagnosis; among those aware, 71.4% were on treatment, and 63.2% were appropriately controlled.

From the longitudinal perspective, only two studies have reported incidence rates of T2DM in our context: one of them found an elevated incidence rate, around 2% per year [21]; whereas the other one reported a similar incidence estimates but also found no urban to rural gradient in T2DM incidences [22]. Nevertheless, this latter manuscript reported higher risk of developing T2DM among those living in high-altitude areas, possibly due to changes in lifestyle and nutrition transition occurring in these settings.

1.4 Alternative methods for T2DM screening

A relevant approach to prevent or delay T2DM complications is to identify those individuals with undiagnosed T2DM [6, 23], though, universal screening for T2DM at the population level is still controversial [24]. Thus, although, the American Diabetes Association recommends T2DM testing for all adults starting at age 45 years regardless of weight, or those who are overweight or obese and have one or more additional risk factor for T2DM [7]; the Disease Control Priorities Group recommends testing individuals at high-risk of T2DM: age≥40 years, individuals with family history of T2DM, obesity, physical inactivity, dyslipidemia, etc [24]. Moreover, recently, the US Preventive Services Task Force has recommended expanding the current criteria for diabetes screening to improve undiagnosed T2DM and dysglycaemia cases [25].

Because of limited economic, human and infrastructure resources in low- and middle-income countries, the identification of cases of T2DM can be better addressed using a two-step approach: in the first step, a risk score – defined as “an objective assessment of the probability of the presence or future development of an adverse health condition” [26]– can be applied to identify subjects at high risk of having or developing T2DM, and, in the second step, a confirmatory test (fasting glucose, OGTT or glycated haemoglobin) can be performed, but only among those categorised as high risk in the previous step [27].

Although the risk scores and neuropathy assessment tools evaluated in this document have been used to detect cases of undiagnosed T2DM and for instance, will be the topic of this thesis, the same methods can be used to consider cases of pre-diabetes and impaired glucose tolerance, potentially evaluable in further work.

1.5 Methods to estimate diagnostic accuracy

According to literature, there are different methods to estimate the diagnostic accuracy and performance of screening methods [28, 29]. Among these, the most simple and familiar techniques are sensitivity and specificity followed by positive and negative predictive values (i.e. including the receiver operating characteristic (ROC) curve) [30]. Thus, the ROC curve is commonly utilised to assess clinical utility for diagnostic models however, evaluation of these models should not only rely on the ROC curve as this technique does not assess both discrimination and calibration [31]. In addition, decision-analytic techniques allow assessment of clinical outcomes but can require new data collection, but decision curve analysis has emerged as a good option [32, 33].

On the other hand, net reclassification indices try to quantify whether a new test provides clinically relevant improvements in prediction; however, these methods have been mainly used for novel blood biomarkers [34]. Besides, a variation of net reclassification index, known as the integrated discrimination improvements have also been suggested as alternative to increase the area under the ROC curve for evaluation the performance of assessment algorithms but for phenotypic or genetic markers [35]. Therefore, the analysis of this thesis will mainly focus in standard diagnostic accuracy techniques as they are simple and understandable for clinicians.

1.6 Risk scores for T2DM screening

Different risk models, also known as risk scores, have been developed to detect T2DM cases. Some of them are useful to detect undiagnosed (prevalent) T2DM cases, whereas other ones predict the development of new (incident) T2DM cases [36]. In addition, blood-free risk scores and those based on self-reported information have been also created to detect cases of undiagnosed T2DM [26].

Many of the existing scores are well-known and widely used but have been mainly developed in high-income countries [37-40]. Among the scores created in LMIC, most of them are from China, India, and other countries of Asia [41-43]. There is, however, great variability in the performance and variables included in risk score for undiagnosed T2DM, supporting the need of developing or at least calibrate/validate a risk score before using them in different regions and contexts.

Of all the risk scores available, to our knowledge, only three models have been developed in Latin America countries, one in Brazil [44], one in Colombia [45], and finally, one in Peru [46].

The Brazilian risk score was created using a specific urban area and fasting glucose as the gold standard [44]. The risk score comprised only three variables (age, body mass index, and known hypertension) and accuracy was moderated (area under the Receiver Operating Characteristic [ROC] of 0.72). The Colombian risk score comprised [45], on the other hand, four variables (age, waist circumference, use of blood pressure medication, and family history of T2DM) in the final model. Using OGTT as the gold standard, the Colombian risk score had also a moderate accuracy for detecting cases of undiagnosed T2DM, with an area under the ROC curve of 0.74. However, results of both risk scores might not be extrapolated, especially in a country with an evident geographical variation such as Peru. Thus, the Peruvian Risk Score was developed by the author of this thesis and is detailed in the next chapter.

Chapter II: The Peruvian Risk Score

In this chapter, the development and performance of the Peruvian Risk Score is detailed as part of the ***first paper for PhD dissertation*** [46], although adapted to the TRIPOD statement [47].

2.1 Source of data

The development of the Peruvian Risk Score entailed two different population-based surveys: the National Survey of Nutritional and Biochemical Indicators for Non-Communicable Diseases (ENINBSC, Spanish acronym, whose data is freely available) [15], and the data of the CRONICAS Cohort Study [48].

2.2 Participants

The ENINBSC is a national population-based survey conducted in Peru (August 2004 and April 2005) to estimate the prevalence of hypertension, T2DM and other risk factors for non communicable diseases at the national and regional level [15]. Potential participants were those aged ≥20 years, habitual residents in the study area, and able to provide consent for participating in the study. Pregnant women and those currently breastfeeding were excluded from the study. The ENINBSC sample was stratified according to Peru's five major regions: Lima, rest of the Coast, urban Highlands, rural Highlands, and Jungle. In each stratum, cluster of blocks were chosen using single random sampling techniques. Within each cluster, a random sample of households and participants was selected.

The CRONICAS Cohort Study is an ongoing cardiopulmonary longitudinal prospective study aimed to estimate the prevalence and incidence of hypertension, T2DM, chronic obstructive pulmonary disease, and obesity in four different settings in Peru differing in terms of urbanicity and altitude: Pampas de San Juan de Miraflores, was the highly-urbanized setting located in Lima, the capital of Peru; Puno in the altitude (3,825 meter above the sea level) contributing with rural and urban areas; and Tumbes, a semi-urban area in the northern coast of Peru [48]. The study started in September 2010 and two follow-up visits were scheduled 15 and 30 months from baseline. A sex- and age-stratified sample was randomly selected for each of the settings and all participants aged ≥35 years, full time residents in the study area, and able to consent, were enrolled. Only baseline and 30-month follow-up data was used for analyses.

2.3 Study procedures

The ENINBSC procedures have been described elsewhere [15]. Briefly, two different visits were scheduled. The first one lasted 40 minutes on average and collected information, applying a face-to-face questionnaire, regarding household characteristics, demographics, lifestyles behaviours, risk factors, as well as blood pressure measurements. The second visit lasted 30 minutes on average and was planned to have an appropriate period of fasting for blood sampling for glucose, lipid profile, and the remaining anthropometric measures (height, weight, and waist circumference) using standardised procedures.

Similarly, the procedures of the CRONICAS Cohort Study have been previously published [48]. Participants responded to a face-to-face questionnaire applied by trained community health workers. Data collected comprised cardiovascular risk factors based on a modified version of the WHO STEP approach questionnaire for surveillance of non-communicable disease [49]. A period of 8 to 12 hours of fasting was required for blood sampling to collect fasting glucose and lipid profile. Height, weight and waist circumference were also assessed, and blood pressure was measured in triplicate after a 5-minute resting period using an automatic monitor (OMRON HEM-780) previously validated in adult's population [50].

2.4 Outcome

In both studies, T2DM was defined as any of the following conditions: fasting glucose ≥7.0 mmol/L (≥126 mg/dL) and/or self-report of physician diagnosis. Fasting glucose was assessed by an enzymatic colorimetric method (glucose oxidase GOD-PAP) in both studies. After excluding individuals aware of disease, undiagnosed T2DM was used to develop and validate the risk score [7].

2.5 Predictors

Variables used to create the risk score were built in similar way in both studies: sex, age (<55, and ≥55 years), education (in years); self-reported smoking (current vs. never/former smoker); alcohol use (user vs. never user); self-reported T2DM in first-degree relatives (participant's parents and/or siblings), and physical activity levels (low vs. moderate/high levels, based on the transport-related domain of the IPAQ). Anthropometric measurements included in the analysis were body mass index ([BMI], <25, 25–29.9, and ≥30 Kg/m^2), waist circumference (<90, 90–99.9, and ≥100 cm), waist to height ratio (<0.50, 0.50–0.59, 0.60–0.69, and ≥070) [51], and hypertension (measured or previously diagnosed) [52].

2.6 Sample size and missing data

A total of 4206 participants were enrolled in the ENINBSC, but only 2,472 were included in the analyses. Reasons for exclusion were: 1524 because age <35 years to make both databases comparable, 129 because no data about fasting plasma glucose levels was available and 81 because known diagnosis of T2DM. In the CRONICAS Cohort Study, 3601 participants were enrolled at baseline but only 2948 records were analysed as 465 had no data about glucose levels, and 188 were excluded because previous diagnosis of T2DM. In addition, data from only 2577 participants was used in the longitudinal assessment of the risk score.

2.7 Statistical analysis methods

Analyses were performed using STATA 13.0 (StataCorp, College Station, TX, US). Firstly, population characteristics of both studies were tabulated using proportions in the case of categorical variables, and mean and standard deviation (SD) in the case of numerical variables. Then, the prevalence and 95% confidence intervals (95% CI) of total T2DM and undiagnosed T2DM were estimated in each study. After that, all cases of known T2DM were excluded from subsequent analyses.

2.7.1 Risk score development

The risk score was derived from the ENINBSC survey taking into account the multistage sampling strategy of the study. Each potential risk factor (i.e. sex, age, family history of T2DM, etc.) was assessed in bivariate models using logistic regression and undiagnosed T2DM as the dependent variable. Then, risk factors with a p-value <0.10 in the bivariate analysis were included in a multiple logistic regression model using stepwise backward elimination with a significance level of 5%. The Hosmer-Lemeshow goodness-of-fit test was used to assess how well the predicted prevalence matched the observed prevalence of undiagnosed T2DM (i.e. p-values over 0.20 indicates that model fits well) [53]. As we sought for an easily applicable and implementable risk score, the risk factors in the final model were each assigned a weighted score by rounding up all regression coefficients in the final model to the nearest integer as in a previous report [38].

For the evaluation of the risk score, the area under the receiver operating characteristic (ROC) curve, as well as sensitivity, specificity, positive and negative predictive values (PPV and NPV) were calculated. The optimal cut-off was determined using the Youden index, a single statistic that captures the performance of a diagnostic test (i.e. sensitivity + specificity – 1) [54]. As one of the main aims of a non-laboratory risk score is to identify people who warrant having a blood test (i.e. FG, OGTT, or HbA1c), the cut-off with the highest sensitivity was also described.

2.7.2 Risk score validation

We assessed the performance of the risk score using bootstrap techniques as well as carrying out an external validation using the CRONICAS Cohort Study. Bootstrapping was utilised to estimate confidence intervals for the area under the ROC curve in our study population. A total of 1,000 random samples with replacement were taken from the development database. The resulting 1,000 prediction models were then assessed to estimate the bootstrap area under the ROC curve using the bias-corrected version of the confidence intervals [55]. In addition, using baseline data from the CRONICAS Cohort Study, validation measures (sensitivity, specificity, predictive values and likelihood ratios) were also estimated.

To evaluate the performance of the risk score, this was compared to previously published models for undiagnosed T2DM: the Brazilian risk score [44], the Qingdao score [56], the Indian risk score [57], the Kuwaiti risk score [58], the patient self-assessment score [38], and the Rotterdam risk score [37] using the c-statistic. Finally, using the follow-up data of the CRONICAS Cohort Study, the risk score was evaluated to detect incident cases of T2DM by excluding those with diabetes diagnosis at baseline.

2.8 Results

Participants from the CRONICAS Cohort Study were, on average, 5 years older, reported consuming lower levels of alcohol, and were less physically active than those from the ENINBSC survey. The characteristics of participants in both studies are detailed in Table II-1.

2.8.1 Prevalence of T2DM and undiagnosed T2DM

In the ENINBSC survey, the T2DM prevalence was 5.1% (129/2538; 95% CI: 4.2%–5.9%), whereas that prevalence was 8.7% (272/3135; 95% CI: 7.7%–9.7%) in the baseline of the CRONICAS Cohort Study. After excluding those with known T2DM, undiagnosed T2DM was present in 2.0% (48/2457; 95% CI: 1.4%–2.5%) in the ENINBSC survey and in 2.9% (85/2948; 95% CI: 2.3%–3.5%) in the CRONICAS Cohort Study.

2.8.2 Development of the risk score

After stepwise backward logistic regression, age, diabetes in first-degree relatives, and waist circumference were independently associated with undiagnosed T2DM (Table II-2). The Hosmer-Lemeshow test showed that the final model fitted relatively well ($p=0.21$). The Peruvian Risk Score was constructed based on the coefficients of that final regression model. The score gave an area under the ROC curve of 0.73 (95% CI: 0.65–0.78), and the optimal cut-off for undiagnosed T2DM using the Youden index was ≥2 (Figure II-1). With this cut-off, about 34.8% of participants were categorised as at high risk of T2DM: sensitivity 69.6%, specificity 65.8%, and PPV and NPV of 3.9% and 99.1% respectively. With a cut-point ≥1, 69.8% of participants would be at high risk of T2DM with improved sensitivity (93.5%) but lower specificity (30.6%). Table II-3 shows the performance of the risk score for detecting undiagnosed T2DM at different cut-offs.

2.8.3 Cross-sectional validation of the risk score

Using bootstrap, the performance of the Peruvian Risk Score was very similar to the obtained in the development model (area under the ROC curve = 0.72; 95% CI: 0.65–0.78). Besides, when the risk score was evaluated using data of the CRONICAS Cohort Study's population, the area under the ROC curve for undiagnosed T2DM was 0.68 (95% CI:

0.62–0.73). At the suggested cut-off of ≥2, 42% would be categorised as undiagnosed T2DM with sensitivity, specificity, PPV and NPV of 70.2%, 58.9%, 4.8%, and 98.5%, respectively (Table II-4).

When previous published algorithms for undiagnosed T2DM were applied to the CRONICAS Cohort Study, the performance of the Rotterdam score ($p<0.001$), Indian score ($p<0.001$), and Qingdao score ($p<0.01$) were poorer than our score; however, our model performed similar to other assessed models, such as the Brazilian risk score ($p=0.93$), the Kuwaiti score ($p=0.26$), and the Patient Self-assessment score ($p=0.74$), but having only three variables.

2.8.4 Longitudinal assessment of the risk score

The performance of this risk score was also assessed to predict incident cases of T2DM using the longitudinal data from the CRONICAS Cohort Study. One hundred twenty one new cases of T2DM were found accounting for 6207 person-years at risk, with an overall incidence of 1.95 (95% CI: 1.63–2.33) cases per 100 person-years of risk. The area under the ROC curve of the score was 0.66 (95% CI: 0.61–0.71). With a cut-off ≥2, 42.5% of participants were categorised as at high risk of developing T2DM: sensitivity, specificity, PPV and NPV were 69.4%, 58.9%, 7.8%, and 97.4%.

2.9 Discussion and limitations

Using a national population-based survey, a simple non-blood risk score, based on age, history of diabetes in first-degree relatives, and waist circumference, was built and shown to perform moderately in detecting undiagnosed T2DM when externally validated. Moreover, the performance of the score was almost similar for detecting incident cases of T2DM in the Peruvian population. This developed risk score does not require a blood test or laboratory services and, for instance, it might be easily implementable in clinical practice. Thus, the Peruvian Risk Score can be potentially self-administered as this asks for general information (age and diabetes in first-degree relatives), and is complemented by a simple anthropometric measure of waist circumference.

According to our results, any patient aged 55 years and above and having at least one first-degree relative with T2DM has greater probability of having undiagnosed diabetes, but also is at risk of developing diabetes in the future. In addition, a greater central obesity, i.e. 100 cm or more, independent of the other terms of the score is alone a good predictor of diabetes as reported in previous studies [51]. Our algorithm included waist circumference instead of body mass index as other risk scores, providing a better indicator of accumulation of visceral fat and metabolic dysfunction in our context [59].

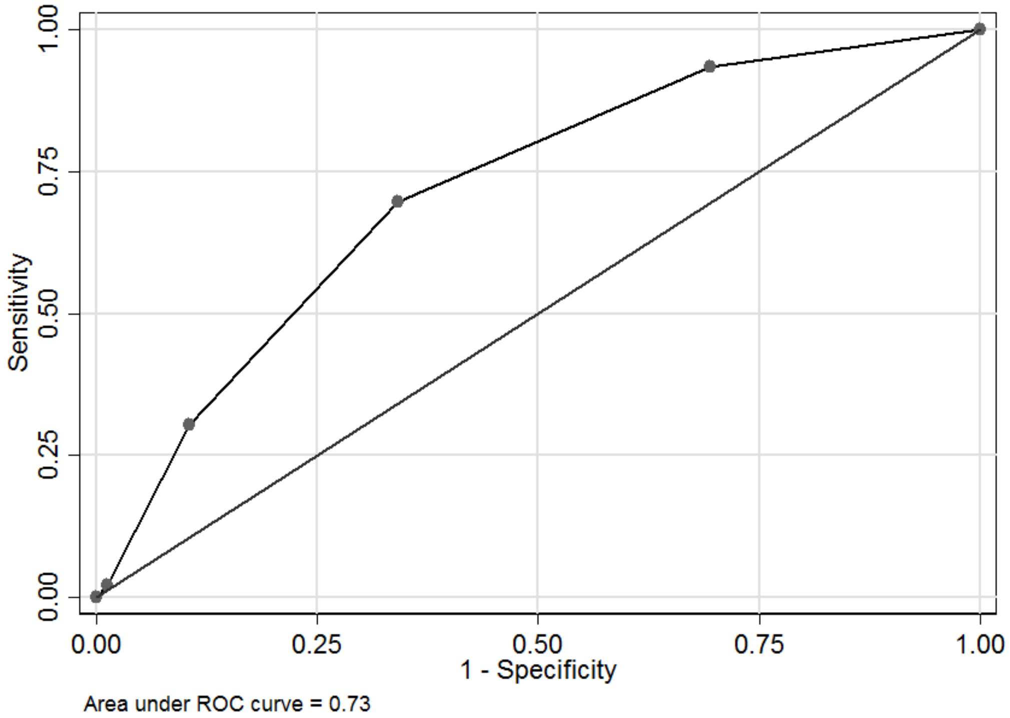
Despite of the moderate performance of the Peruvian Risk Score, some limitations need to be highlighted. The OGTT was not used as gold standard for T2DM diagnosis as it is not usual to be performed in epidemiological studies. Thus, the Peruvian Risk Score need to be evaluated or calibrated appropriately. In addition, as secondary databases were used to create the risk score model, information regarding diet patterns and history of gestational T2DM among women, was not evaluated. Finally, the model was based on the idea of risk stratification instead of individualization; thus, numerical variables were categorised instead of being preserved in their original form. Nevertheless, the objective of the original paper was to develop a simple and easily applicable score to detect undiagnosed T2DM.

2.10 Further steps and implications

There is a need of assessing existing risk scores for undiagnosed T2DM screening at the population level in resource-constrained settings such as Peru. Moreover, there is limited information in Latin American countries evaluating the performance of risk scores using OGTT as the gold standard. From the perspective of LMIC, these risk scores should include objective and easily evaluable measurements.

As other risk models, the Peruvian risk score needs to be assessed before it can be used in other populations. Thus, further scrutiny, using OGTT as gold standard should be guaranteed. In addition, the inclusion of other variables in the model requires a more detailed assessment of sociodemographic, but specially, lifestyle behaviours and anthropometric characteristics of participants.

Figure II-1: Receiver operating characteristic (ROC) curve for the risk score in predicting undiagnosed T2DM: Development database



The area under the ROC curve was 0.73 (95% CI: 0.65 – 0.78) for the risk score.

Table II- 1: Sociodemographic characteristics of participants without history of T2DM according to study

	ENINBSC Study (n = 2,472)	CRONICAS Study (n = 2,945)
Demographic variables		
Sex (% females)	1,209 (48.9%)	1,500 (50.9%)
Age [mean (SD)]	50.5 (12.1)	55.3 (12.7)
Education in years [mean (SD)]	7.8 (4.9)	8.0 (4.9)
Behavioural variables		
Current smoking (%)	391 (15.9%)	369 (11.5%)
Alcohol use (%)	2,323 (94.1%)	1,600 (54.3%)
Family history of diabetes (%)	268 (11.2%)	351 (11.9%)
Physical activity (% low level)	606 (24.5%)	938 (31.9%)
Anthropometric measures		
Body mass index [mean (SD)]	25.7 (4.5)	27.6 (4.6)
Waist circumference [mean (SD)]	91.0 (11.4)	91.5 (11.0)
Waist-to-height ratio [mean (SD)]	0.58 (0.08)	0.59 (0.07)
Systolic blood pressure [mean (SD)]	114.5 (18.5)	117.2 (18.9)
Diastolic blood pressure [mean (SD)]	71.1 (11.9)	73.4 (11.1)
Hypertension (%)	579 (23.8%)	705 (24.0%)
Total cholesterol [mean (SD)]	174.2 (36.9)	199.7 (39.6)
HDL cholesterol [mean (SD)]	43.5 (5.3)	41.7 (11.5)

SD = standard deviation, HDL = high-density lipoprotein
*Results may not add due to missing values

Table II-2: Risk factors and beta coefficients for undiagnosed T2DM: Final regression model using ENINBSC database (N = 2,367)

	Crude model	**Final model***		**Score**
	OR (95% CI)	**β (SE)**	**OR (95% CI)**	
Sex				
Male (vs. female)	0.68 (0.38 – 1.21)			
Age				
≥55 (vs. <55 years)	2.05 (1.16 – 3.64)	0.61 (0.18)	1.85 (1.30–2.63)	1 (vs. 0)
Current smoking				
Current (vs. never/formers)	0.34 (0.11 – 1.12)			
Alcohol user				
User (vs. never user)	1.46 (0.34 – 6.27)			
Diabetes in relatives				
Yes (vs. no)	2.90 (1.48 – 5.66)	0.85 (0.42)	2.34 (1.04–5.31)	1 (vs. 0)
Physical activity				
Low (vs. moderate/high level)	2.24 (1.25 – 4.01)			
Body mass index				
Overweight (vs. normal)	1.07 (0.54 – 2.13)			
Obese (vs. normal)	2.23 (1.11 – 4.49)			
Waist circumference				
90.0 to <99.9 cm (vs. <90 cm)	1.93 (0.91 – 4.10)	0.74 (0.33)	2.09 (1.09–4.02)	1 (vs. 0)
100+ cm (vs. < 90 cm)	4.10 (1.99 – 8.44)	1.40 (0.23)	4.07 (2.60–6.40)	2 (vs. 0)
Waist-to-height ratio				
0.50 – 0.59 (vs. <0.50)	1.41 (0.41 – 4.86)			
0.60 – 0.69 (vs. <0.50)	2.97 (0.88 – 10.0)			
0.70+ (vs. <0.50)	4.84 (1.27 – 18.5)			
Hypertension				
Yes (vs. no)	1.68 (0.91 – 3.09)			

* The model was created using backward elimination from the initial full model until we reached a final model with statistically significant covariates.

Table II-3: Performance of different cut-offs for detecting undiagnosed T2DM in the development database

Total score	At high risk*	Sensitivity	Specificity	PPV	NPV	Correctly classified	LR+	LR-
≥ 1	69.8%	93.5%	30.6%	2.6%	99.6%	31.8%	1.34	0.21
≥ 2	34.9%	69.6%	65.8%	3.9%	99.1%	65.9%	2.04	0.46
≥ 3	11.0%	30.4%	89.4%	5.4%	98.5%	88.3%	2.87	0.78
≥ 4	1.3%	2.2%	98.7%	3.2%	98.1%	96.8%	1.68	0.99

PPV = Positive predictive value; NPV = Negative predictive value; LR+ = Positive likelihood ratio, LR- = Negative likelihood ratio
* Those at high risk are the proportion of participants over the total score.

Table II-4: Performance of different T2DM risk scores compared to Peruvian Risk Score using the CRONICAS Study (validation sample)

Method (proposed cutoff)	# of variables	AUC	Sensitivity	Specificity	PPV	NPV	LR+	LR-
Brazilian risk score (≥18)	3	0.65	66.7%	61.9%	4.9%	98.4%	1.75	0.54
Qingdao risk score (≥17 & ≥14)*	4	0.58	83.3%	33.3%	3.6%	98.5%	1.25	0.50
Indian risk score (≥21)	5	0.54	94.0%	15.5%	3.1%	98.9%	1.11	0.39
Kuwaiti risk score (≥32)	4	0.62	45.2%	78.4%	5.8%	98.0%	2.09	0.70
Patient self-assessment score (≥5)	6	0.64	61.4%	66.8%	5.1%	98.3%	1.85	0.58
Rotterdam risk score (≥36)	6	0.55	94.0%	16.8%	3.2%	99.0%	1.13	0.35
Peruvian risk score (≥2)	3	0.68	70.2%	58.9%	4.8%	98.5%	1.71	0.51

AUC = Area under the ROC curve; PPV = Positive predictive value; NPV = Negative predictive value; LR+ = Positive likelihood ratio, LR- = Negative likelihood ratio
* Different cut-offs for males (≥17) and females (≥14).

Chapter III: EZSCAN for undiagnosed T2DM: a systematic review and meta-analysis

3.1 Background

In addition to the existence of risk score models, there are devices focused on assessing autonomic dysfunction, as a way to increase the probability to detect cases or individuals at risk of T2DM. Autonomic dysfunction is an early, and many times subclinical, consequence of hyperglycaemia. Diabetic autonomy neuropathy is one of the least recognized complications of T2DM, but it can be of clinical significance due to cardiovascular, gastrointestinal, sudomotor, and ocular autonomic neuropathy complications [60].

There are different tests to assess autonomic dysfunction but usually require well-trained health staff, some of them are time consuming, and require active patient participation. As early damage of nerves can be found since the onset of T2DM [61], some devices has emerged to assess small-fibers autonomic dysfunction [62]. The EZSCAN, developed by Impeto Medical (Paris, France), is a non-invasive device that, based on sudomotor function assessment, may help to detect both, cases of undiagnosed T2DM and cases at risk of developing T2DM.

As the EZSCAN requires minimal training required and obtained results are not human dependent, this chapter is focused in a systematic review and meta-analysis conducted to assess the performance (i.e. area under the ROC curve, sensitivity and specificity) of the EZSCAN for detecting undiagnosed T2DM cases (**Second paper for PhD dissertation**) [63].

3.2 Eligibility criteria

We searched for observational studies assessing the diagnostic accuracy of the EZSCAN for undiagnosed T2DM, conducted in different parts of the world, but reported in English. Studies were excluded if they were only abstracts or review articles, enrolled individuals aged <18 years or cases with type 1 diabetes mellitus, and defined type 2 diabetes mellitus (T2DM) by using blood markers other than OGTT or FG (i.e. HbA1c). The rationale for this decision was based on discrepancies between HbA1c and glycaemia in different racial and ethnic groups and that HbA1c is not commonly used for undiagnosed T2DM.

3.3 Information sources and searches

A comprehensive literature search using the Ovid database (PubMed-Medline, Embase, Global Health, and Health Management Information Consortium) as well as CINAHL, and SCOPUS, until March 29, 2017, was conducted. The following keywords were utilised for the systematic searching: type 2 diabetes mellitus, hyperglycaemia, EZSCAN, SUDOSCAN, and sudomotor function [62]. The term SUDOSCAN was also included in the search strategy as it uses the same principle (i.e. sudomotor function assessment) for detecting diabetic neuropathy [64, 65]. The search strategy of Ovid is available in Table III-1. The Impeto Medical website was also searched to find other published manuscripts [66].

3.4 Study selection, data extraction and quality assessment

Titles and abstracts of retrieved articles were reviewed independently by two investigators to select potentially relevant articles, and disagreements were discussed and solved by consensus. Using a standardised data extraction form, we collected information on lead author, publication year, country, study design, inclusion criteria, used gold standard, sample size, mean age, percentage of male participants, and different indicators of the performance of the EZSCAN to detect undiagnosed T2DM (outcome, area under the ROC curve, cut-off, sensitivity, specificity, among others).

Quality assessment of individual studies was performed to identify potential sources of bias and to limit, if possible, the effect of these biases on the conclusions of the review. For this, the Revised Version of the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) checklist was used [67]. This tool included risk of bias assessment (i.e. participant selection, index test, reference standard, and flow and timing) as well as applicability.

3.5 Synthesis of results and meta-analysis

The primary outcome of interest was undiagnosed T2DM (i.e. newly-diagnosed T2DM) identified by OGTT or FG. Secondary outcomes included other glucose metabolism disorders such as impaired glucose tolerance, impaired fasting glucose and, dysglycaemia.

Statistical analyses were performed using Stata version 13 for Windows (StataCorp, College Station, TX, US). For report purposes the Preferred Reporting Items for Systematic Review and Meta-analysis of Diagnostic Test Accuracy Study (PRISMA-DTA) was used [68] as well as the Cochrane Handbook for Diagnostic Test Accuracy Reviews [69]. Initially, the studies included in the systematic review were described, including: publication year, country, study design, inclusion criteria, gold standard, sample size, mean age, and proportion of males. In addition, the performance of the EZSCAN in each study was tabulated, and the area under the receiver operating characteristic (ROC) curve, best cut-off, sensitivity, and specificity, and their respective 95% confidence intervals (95%CI) were reported, if available.

A meta-analysis of the performance of the EZSCAN was conducted using data from studies with undiagnosed T2DM as outcome. Information used in meta-analysis was taken as proposed by manuscripts according to the best EZSCAN threshold cut-off reported. The "*metaprop"* command in STATA was used to estimate sensitivity, specificity and positive (PPV) and negative (NPV) predictive values and their respective 95% CI [70]. The "*metaprop*" command obtains a pooled estimate as a weighted average, by fitting the logistic-normal random-effects model without covariates but random intercepts. The pooled estimate was then calculated using the Freeman-Tukey Arcsine Transformation to stabilize variances as suggested in literature [71]. In addition, a graph containing the plot of the Hierarchical Summary Receiver Operating Characteristic (HS-ROC) model [72], a summary point of sensitivity and specificity and the 95% confidence region for that point was obtained by using the "*metandiplot*" command [73]. Heterogeneity of estimates and 95%CI was determined using the I^2 measure [74].

3.6 Results

3.6.1 Selection and characteristics of studies

A total of 1461 citations were identified through our systematic search, with a further 16 citations identified using the Impeto Medical website. After excluding duplicates (n=330), a total of 1147 citations were independently screened, of which 31 were retrieved for detailed assessment (agreement between reviewers, 97.2%, kappa = 0.61, p<0.001). Of the 31 revised manuscripts, 27 did not fit our inclusion criteria (Figure III-1); therefore, four studies were included in the systematic review.

The characteristics of the studies included in the systematic review are shown in Table III-2. All the four studies were cross-sectional in nature. A total of 7720 individuals were included from all the studies, but 5824 subjects came from a single study [75]. This latter study enrolled individuals from the general population, whereas the remaining three studies recruited participants at clinics, mainly individuals going for healthy check-ups.

3.6.2 Risk of bias

Overall, participant selection bias was present in 3 out of 4 of the studies included in the meta-analysis [76-78]: individuals under healthy check-ups were enrolled in the original studies (Table III-3). In addition, flow and timing was unclear in the same three studies, and OGTT was not used as gold standard in one of the four studies [78].

3.6.3 Meta-analysis: EZSCAN performance for undiagnosed T2DM

Undiagnosed T2DM was the outcome of interest in the four studies (Table III-4). Other outcomes evaluated in these papers included impaired glucose tolerance [76, 77], impaired fasting glucose [78], and dysglycaemia [75].

When undiagnosed T2DM was the outcome, only two studies reported results of area under the ROC curve ranging from 53% to 73% [76, 78]. In addition, only two studies used 50% as the suggested EZSCAN cut-off for undiagnosed T2DM screening [76, 77], whereas one used 34% [78], and the last one utilised 30% [75]. Sensitivity varied from 53% to 81%, whilst specificity ranged from 43% to 70%. Finally, positive predictive values (PPV) varied from 10% to 40%, whereas negative predictive values (NPV) ranged from 71% to 98%.

When using HS-ROC (Figure III-2), summary sensitivity was 72.0% (95%CI: 60.0% – 83.0%), specificity was 56.0% (95%CI: 38.0% – 74.0%), PPV was 24% (95%CI: 12.0% – 37.0%), and NPV was 89% (95%CI: 82.0% – 97.0%). In addition, positive and negative likelihood ratios were 1.68 (95%CI: 1.35 – 2.10) and 0.48 (95%CI: 0.36 – 0.66), respectively, whereas the DOR was 3.49 (95%CI: 2.18 – 5.57). Heterogeneity for sensitivity was 79.2% (95%CI:

44.0% – 92.0%), whereas for specificity was 99.1% (95%CI: 98.5% – 99.6%).

3.7 Discussion and limitations

According to the results of this systematic review and meta-analysis, the performance of the EZSCAN in the detection of undiagnosed T2DM cases can be considered moderately acceptable especially in the case of sensitivity, and even comparable to different well-known T2DM risk scores [37, 40]. To put in context our findings, the sensitivity of HbA1c, using a cut-off ≥6.5% (48 mmol/mol), for detecting undiagnosed diabetes was 52.8% using OGTT as the gold standard [12]. Thus, apparently, the EZSCAN might perform better that HbA1c although other studies are needed to corroborate these findings.

There are, however, some limitations that need to be highlighted. First, there is a risk of bias based on participant selection that can complicate extrapolation of results: many of the studies were performed in clinical context (i.e. clinical check-ups) instead of using population level assessments. Second, a high level of heterogeneity between studies was found (greater than 75%) in all estimations (i.e. sensitivity, specificity, etc). Since a small number of studies were included in the meta-analysis; results need to be cautiously interpreted despite of the fact that random effect models were used in calculations [79]. In addition, heterogeneity in results of the EZSCAN performance can be secondary to characteristics of the context and individuals: predictive values as well as likelihood ratios can depend on baseline risk of evaluated subjects. For example, the association of body mass index –one of the variables used in scoring individuals through EZSCAN– with the risk of diabetes may vary in different populations [80], and explain variability found in this report. Third, characteristics of the study population were poorly reported and this is reflected in the quality assessment. As all the studies assessing EZSCAN were recently published (from 2010 and onwards); authors should have been utilised the Standards for Reporting Diagnostic Accuracy Studies (STARD) to guide their manuscripts' writing [81, 82]. Future studies should follow these guidelines to guarantee an appropriate reporting of diagnostic studies. Fourth, as the EZSCAN is a commercial device, underlying algorithms for estimating the risk of T2DM are not freely available; and for instance, they are unknown. Finally, given the limited number of studies assessed, EZSCAN threshold was not meta-analysed as the performance of the diagnostic test depends on the population in which the test is used. Thus, for our analyses, pooled sensitivity and specificity were calculated using the best cut-off reported by studies and not the same in all cases. In addition, there is limited data evaluating the potential impact of EZSCAN for undiagnosed T2DM at the population level. Future studies should be focused on population-based samples instead of referral health facilities, but also in different ethnic groups as only studies from China and India were used in this review. A study from Mexican population was also included in the meta-analysis, but the selection of the sample was biased and FG was used as gold standard [78]. Moreover, as the number of studies included in the analysis was small, publication bias was not assessed (usual tests for publication bias are underpowered when <10 studies are evaluated).

3.8 Update of the systematic review and meta-analysis

As April 22, 2018, a new manuscript assessing the performance of the EZSCAN as a screening tool for undiagnosed T2DM in Chinese individuals was published in May 2017 [83]. Subjects were recruited in a third-level hospital as part of a routine health check. OGTT was used for detecting cases of undiagnosed T2DM, excluding those with previous diagnosis of T2DM or pre-diabetes. A total of 6270 subjects were enrolled, 63.1% males. The area under the ROC curve was 81.3% (95% CI: 78.4% - 84.2%), with an empirical cut-off of 44.5, and a sensitivity and specificity of 73.2% and 83%, respectively. Thus, results of this manuscript were included in a new re-assessment of the meta-analysis (HS-ROC).

Using HS-ROC (See Figure in Appendix C), summary sensitivity was 73.6% (95% CI: 65.6% - 80.3%), specificity was 63.2% (95% CI: 49.1% - 75.4%), PPV was 28.0% (95% CI: 16.0% - 40.0%), and NPV was 92.0% (95% CI: 88.0% - 95.0%). In addition, positive and negative likelihood ratios were 2.00 (95% CI: 1.40 – 2.86) and 0.42 (95% CI: 0.31 – 0.57), respectively, whereas the DOR was 4.80 (95% CI: 2.60 – 8.87). Heterogeneity for sensitivity was 79.0%, whereas for specificity was 99.0%.

On the other hand, when the only study using FG was excluded from analyses [78], summary sensitivity was 73.0% (95%CI: 62.8% – 81.3%), summary specificity was 61.3% (95%CI: 44.0% – 76.2%), whereas PPV was 33.0% (95%CI: 24.0% – 43.0%), NPV was 89.0% (95%CI: 84.0% – 94.0%), positive likelihood ratios was 1.89 (95% CI: 1.24 – 2.88), negative predictive value was 0.44 (95% CI: 0.30 – 0.65), and the DOR was 4.30 (95% CI: 2.02 – 9.15). See Figure in Appendix D.

3.9 Other potential methods for T2DM screening

In addition to the EZSCAN, other devices to assess neuropathy dysfunction can have an impact on T2DM screening: pupillometry and biothesiometry.

3.9.1 Pupillometry for T2DM screening

Although there are different manuscripts assessing the differences in pupil parameters between T2DM cases (with and without complications) and apparently healthy subjects [84-90], quite a few has focused on the potential of the pupil parameters for detecting underdiagnosed T2DM cases [91]. Several methods have been described to evaluated pupil size and reflex among T2DM cases, including a great number of parameters, mainly explained because the static and dynamic characteristics of the pupil function. Thus, to assess the static characteristics of the pupil, only a camera (and then callipers) or a portable pupillometer is needed. However, to evaluate the pupil dynamics, in addition to the pupillometer, a computer and software are needed to appropriately interpret and obtained results. Moreover, only some of the parameters can be obtained using static pupillometry, but when software is utilised, a great number of pupil parameters can be easily and fast acquired after some minutes of darkness-adaptation, as well as after diverse light stimuli strategies (1 flash, 25 flashes, etc) [92].

The most common parameters reported in the literature when comparing T2DM cases and apparently healthy individuals were latency time to pupil constriction (dynamic pupillometry) [86, 87, 89], and pupil diameter and pupil area (static pupillometry) [85, 87, 93]. Overall, manuscripts reported differences in diverse pupil indicators when comparing populations of interest, yet these pupillometry parameters have been used as a screening tool for T2DM neuropathy instead of undiagnosed T2DM [88, 90].

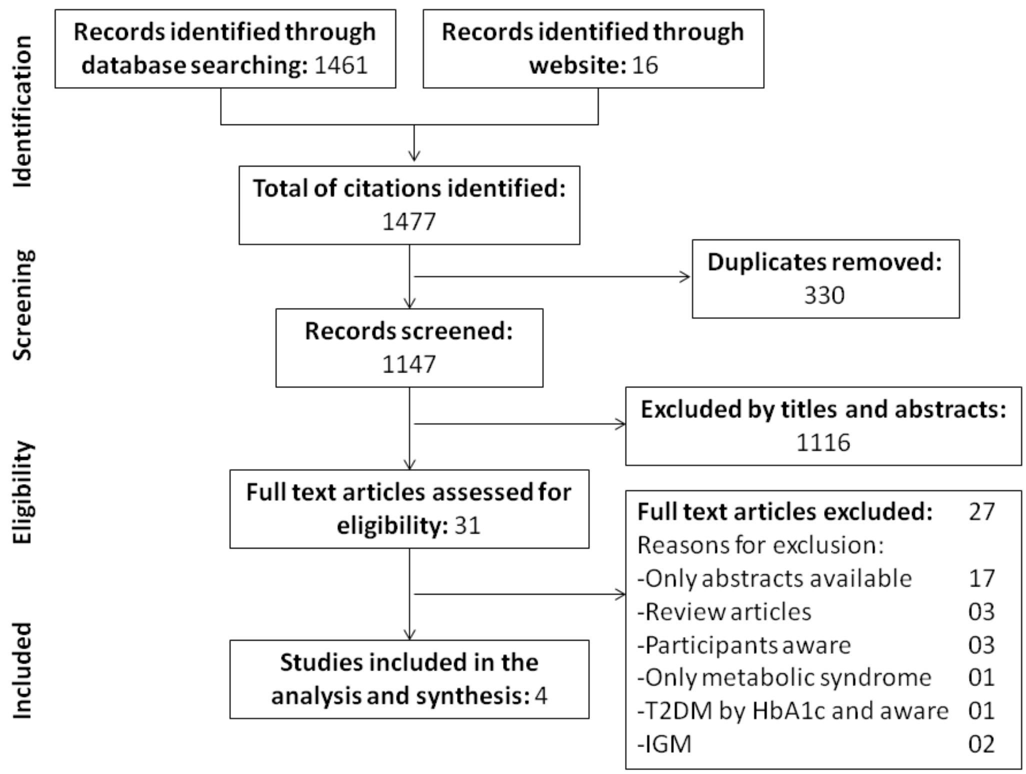
In Peru, *Lerner et al* [91], using dynamic pupillometry, found that diagnostic accuracy of several pupil measurements were fair enough for T2DM screening with an area under the ROC curve ≥0.60. Of these parameters, pupil diameter and amplitude of pupil reaction were those with better performance. Nevertheless, the paper focused on differences in pupil measurements comparing individuals with and without T2DM, and not for undiagnosed cases. In addition, a hospital-based sample was used instead of a population-based sampling.

3.9.2 Biothesiometry in T2DM

The biothesiometer is a device used to assess the threshold of appreciation of vibration in human subjects (vibration perception threshold) [94]. Although this tool can be utilised in different neurological diseases [95], the use of biothesiometry in T2DM has been restricted to the screening of diabetic neuropathy among T2DM cases [96]. In fact, to our knowledge, there is no study reporting the usability of biothesiometry in the screening of undiagnosed T2DM. As previously mentioned, small nerves damage occurred in early stages of T2DM, and even before the diagnosis of the disease; as a result, this study will take advantage on that and assess the performance of biothesiometry as a screening tool for undiagnosed T2DM cases.

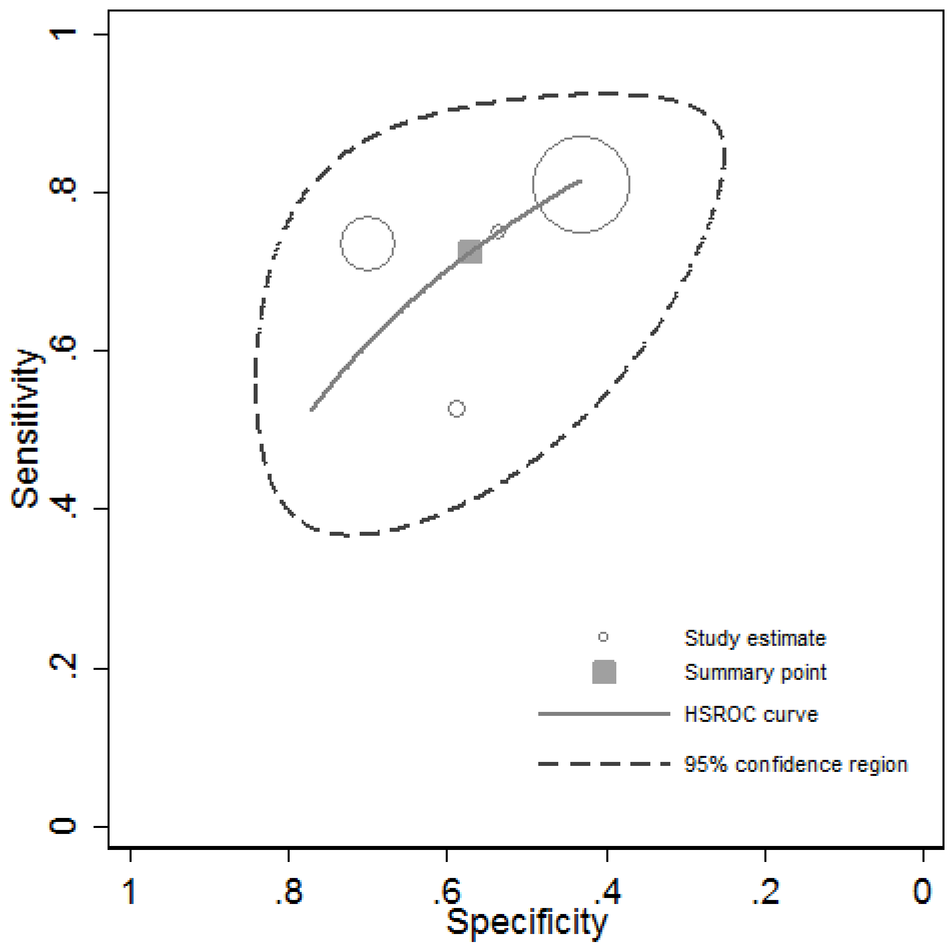
Overall, the performance of different devices based on neurological assessment and function, such as EZSCAN, pupillometry and biothesiometry for screening of T2DM need to be evaluated, including the potential combination of these devices with risk score models (i.e. anthropometric measurements or sociodemographic information). The form to combine risk scores and neuropathy assessment tools can be easily conducted by using basic operations of Boolean algebra [97], with a potential improving in sensitivity if using disjunction terms (alternative logic: OR) instead of conjunction terms (sequential logic: AND).

Figure III-1: Flowchart of database searches and articles included in the systematic review.



T2DM: Type 2 diabetes mellitus, HbA1c = glycated haemoglobin, IGM = impaired glucose metabolism.

Figure III- 2: Performance of EZSCAN in the screening of T2DM: Meta-analysis using HSROC.



Sensitivity = 72.0% (95%CI: 60.0% - 83.0%); specificity = 56.0% (95%CI: 38.0% - 74.0%); likelihood ratio positive = 1.68 (95%CI: 1.35 – 2.10); likelihood ratio negative = 0.48 (95%CI: 0.36 – 0.66); DOR = 3.49 (95%CI: 2.18 – 5.57). HSROC curve is shown only for sensitivities and specificities at least as large as the smallest study-specific estimates.

Table III-1: OVID search strategy for EZSCAN

Databases included:
Global Health 1910 to 2017 Week 11; HMIC Health Management Information Consortium 1979 to January 2017; Journals@Ovid Full Text March 29, 2017; Ovid MEDLINE(R) 1946 to March Week 4 2017; PsycINFO 1806 to March Week 3 2017; Embase 1974 to 2016 March 29.

#	Searches	Results
1	type 2 diabetes.mp.	382,642
2	diabet*.mp.	2,257,087
3	hyperglycem*.mp.	225,801
4	T2D*.mp.	69,504
5	DBM.mp	4,657
6	(#1 or #2 or #3 or #4 or #5)	2,329,381
7	exp Diabetes Mellitus	1,249,251
8	exp Diabetes Mellitus, Type 2/	311,830
9	(#7 or #8)	1,249,251
10	(#6 or #9)	2,335,634
11	EZScan.mp.	81
12	SUDOSCAN.mp	167
13	sudom*.mp	4,932
14	(#11 or #12 or #13)	5,021
15	**(#10 and #14)**	**1,345**

Table III-2: Characteristics of the studies included in the systematic review

Study, publication year	Country	Study design	Inclusion criteria	Gold standard	Size	Mean age	% male
Chen X, 2015 [76]	China	Cross-sectional	Subjects in routine health check visiting a Community Hospital, at risk of T2DM (age ≥ 45 years).	OGTT	270	58.6	32%
Ramachadran A, 2010 [77]	India	Cross-sectional	Individuals in specific clinics aged between 21-75 years.	OGTT	212	43.4	45%
Sanchez-Hernandez O, 2015 [78]	Mexico	Cross-sectional	Individuals recruited in a clinic in Mexico; ≥18 years, apparently healthy and attending a full check-up.	FG	1,414	44.7	50%
Yang Z, 2013 [75]	China	Cross-sectional	Individuals from two communities in Shanghai aged 40+ years.	OGTT	5,824	58.3	40%

FG = fasting glucose; OGTT = oral glucose tolerance test.

Table III-3: Quality assessment of the studies included in the systematic review (QUADAS-2)

Study, publication year	Risk of bias				Applicability		
	Patient selection	**Index test**	**Reference standard**	**Flow and timing**	**Patient selection**	**Index test**	**Reference standard**
Chen X, 2015 [76]	High	Low	Low	Unclear	Unclear	Low	Low
Ramachadran A, 2010 [77]	High	Low	Low	Unclear	High	Low	Low
Sanchez-Hernandez O, 2015 [78]	High	Unclear	High	Unclear	High	Unclear	High
Yang Z, 2013 [75]	Low	Low	Low	Low	Low	Low	Low

Table III-4: Performance of the EZSCAN in the studies included in the systematic review

Study, publication year	Outcome	AUC	Cut-off	Sensitivity	Specificity
Chen X, 2015 [76]	IGT	78% (72% - 83%)	37%	82% (72% - 90%)	63% (55% - 71%)
Chen X, 2015 [76]	T2DM	53% (43% - 62%)	50%	53% (36% - 69%)	59% (47% - 70%)
Ramachadran A, 2010 [77]	IGT	--	50%	70% (not reported)	54% (not reported)
Ramachadran A, 2010 [77]	T2DM	--	50%	75% (not reported)	54% (not reported)
Sanchez-Hernandez O, 2015 [78]	IFG	65% (not reported)	27%	69% (not reported)	56% (not reported)
Sanchez-Hernandez O, 2015 [78]	T2DM	73% (not reported)	34%	73% (not reported)	70% (not reported)
Yang Z, 2013 [75]	IFG, IGT or T2DM	--	30%	73% (71%-75%)	46% (45%-48%)
Yang Z, 2013 [75]	T2DM	--	30%	81% (78%-83%)	43% (42%-44%)

IFG = Impaired fasting glucose; IGT = Impaired glucose tolerance; T2DM = type 2 diabetes mellitus; AUC = area under the curve. Values in brackets are 95% confidence intervals (95%CI).

Chapter IV: Materials and Methods

In this chapter, we describe the research question, objectives and hypotheses, as well as materials and methods utilised during fieldwork activities.

4.1 Research question

What is the diagnostic accuracy of existing blood-free risk scores and neuropathy assessment tools (i.e. EZSCAN, pupillometer and biothesiometer) to detect cases of undiagnosed T2DM at the population level?

4.2 Primary objectives

4.2.1 Primary objective 1:

To assess the diagnostic accuracy of blood-free risk scores and neuropathy assessment tools to detect cases of undiagnosed T2DM at the population level in a semiurban area in Peru.

Hypothesis 1: Using the area under the ROC curve, we expect a sensitivity of at least 75% compared to the oral glucose tolerance test.

4.2.2 Primary objective 2:

To compare the diagnostic accuracy of the EZSCAN and other neuropathy assessment devices and existing blood-free risk scores to detect cases of undiagnosed T2DM in a semiurban area in Peru.

Hypothesis 2: We hypothesised that EZSCAN will have a better diagnostic accuracy compared to the other devices (pupillometer and biothesiometer), and its performance will be similar to other blood-free risk scores (Finnish

Diabetes Risk Score – FINDRISC, the Latin America FINDRISC, and the Peruvian Risk Score).

4.2.3 Primary objective 3:

To evaluate, if possible, the performance of a combination of blood-free risk scores and neuropathy assessment devices for detecting cases of undiagnosed T2DM in a population-based sample of a semiurban area in Peru.

Hypothesis 3: We hypothesised that this combination, using Boolean algebra, will have at least a sensitivity of 75% compared to oral glucose tolerance test.

4.3 Secondary objectives

In addition to the aforementioned objectives, this document will also focus on:

Secondary objective 1: To determine the prevalence of T2DM and undiagnosed T2DM on a population-based sample of a semiurban area in Peru.

Secondary objective 2: To evaluate the performance of the EZSCAN for detecting cases of undiagnosed T2DM in a population-based sample of a semiurban area in Peru.

Secondary objective 3: To evaluate the performance of pupil parameters using static pupillometry for detecting cases of undiagnosed T2DM in a population-based sample of a semiurban area in Peru.

Secondary objective 4: To evaluate the performance of the biothesiometer for detecting cases of undiagnosed T2DM in a population-based sample of a semiurban area in Peru.

Secondary objective 5: To assess the performance of some of the existing blood-free risk scores (FINDRISC and Peruvian Risk Score) for detecting cases of undiagnosed T2DM in a population-based sample of a semiurban area in Peru.

4.4 Methods

4.4.1 Study design and setting

A population-based cross-sectional study was conducted enrolling a random sample of participants from Tumbes, a semiurban area in the north of Peru (See Figure IV-1 for detailed location). According to projections of the last national census, Tumbes has 243,000 inhabitants in an area of 4,670 km^2 [98]. Approximately 61% of inhabitants have a health insurance, 80% of households have drinking water, and life expectancy is 75 years. As semiurban area, the setting comprises traditional agricultural and fishing villages intermixed with rapidly growing urban sections.

The rationale for selecting this setting was because prevalence of obesity, by body mass index (32% vs. 18%), and T2DM, by fasting plasma glucose (10% vs. 7%), is over the national average [17]. Besides, data of the CRONICAS Cohort Study shows a relatively high population-level incidence of T2DM (2.0; 95%CI: 1.6 – 2.3) per 100 person-years), as high as in Lima, the largest and most urbanized region in Peru [22].

4.4.2 Participants and sampling

Eligible participants were those aged between 30 and 69 years, full time resident in the study area (i.e. ≥6 months) and able to understand procedures and provide informed consent. Women that reported being pregnant or individuals having any physical disability preventing anthropometric measurements (weight, height, blood pressure or waist circumference) or those bedridden were excluded from the study.

Secondary selection criteria were also applied depending on the device assessment tool used. For EZSCAN evaluation, exclusion criteria involved the self-report of use of implantable electrical devices (i.e. pacemaker) or known sensitivity to nickel as the standard electrodes are made of that material [99, 100]. For pupillometry evaluation, those participants who self-reported having severe neurological conditions (i.e. Parkinson's disease, Alzheimer's diseases or multiple sclerosis) or those with ocular complications such as corneal lesions, glaucoma or severe cataracts, were excluded from the study. The reason for exclusion was based on the fact that these conditions might interfere with the proper interpretation of pupillometry results [91].

A sex-stratified, single-stage random sampling strategy was conducted using the most updated census available in the study area (2014). To avoid potential clustering of behavioural factors, only one participant per household was invited to participate in the study.

4.5 Test methods

4.5.1 Reference standard

Undiagnosed T2DM was the outcome variable of interest. The variable was defined according to the World Health Organization threshold using the OGTT. For our purposes, individuals who were not aware of having T2DM diagnosis and had fasting glucose level ≥126 mg/dL (≥7.0 mmol/L) or 2-hour plasma glucose ≥200 mg/dL (≥11.1 mmol/L) were classified as undiagnosed T2DM.

In addition, those with 2-hour plasma glucose ≥126 mg/dL (≥7.8 mmol/L) and <200 mg/dL (<11.1 mmol/L), but fasting plasma glucose <126 mg/dL (<7.0 mmol/l) were classified as having impaired glucose tolerance. On the other hand, impaired fasting glucose was defined as fasting plasma glucose ≥110 mg/dL (≥6.1 mmol/L) but <126 mg/dL (<7.0 mmol/L), and 2-hour plasma glucose <140 mg/dL (<7.8 mmol/L). Finally, dysglycaemia was defined as the presence of impaired fasting glucose, impaired glucose tolerance or T2DM [7].

4.5.2 Index tests

Two groups of variables were used as index test of interest: those related to autonomic devices and those related to risk scores for undiagnosed T2DM.

Autonomic devices:

EZSCAN^{TM}: It is a non-invasive device developed to identify individuals at increased risk of T2DM (Impeto Medical, Paris, France). The evaluation does not require fasting and results reproducibility are supposed to be better than glycaemia indicators [100]. The EZSCAN device is based on the fact that small fiber neuropathies are common in people with insulin resistance and pre-diabetes [101]. The device assesses sweat gland function by applying a small direct current in both hands and feet to measure chloride ions conductance [100]. The EZSCAN process evaluates sweat gland function in relation to sweat innervations and results are derived by using complementary individual information (height, weight, sex and age). Results are expressed in both colours: green (no risk), yellow (moderate risk, orange (high risk), and red (very high risk), and percentages with pre-specified values: no sweat dysfunction (<50%), median sweat dysfunction (50%-65%), and high sweat dysfunction (>65%). For study purposes, EZSCAN results will be used as continuous variables to assess suggested provider's cut-off or define appropriately a cut-off for our population.

Pupillometer: Human pupil size varies, on average, from 1 mm to 9 mm. Pupil reflex is mediated by acetylcholine and nor-adrenaline, causing miosis and mydriasis, respectively. Changes in pupil size in response to light stimulus is based on a functional equilibrium between sympathetic and parasympathetic activity [102]. Small-fiber dysfunction has been reported even from impaired fasting glucose [103]; and as a result, it is expectable to find differences in pupil diameter between undiagnosed T2DM and individuals without T2DM. Measurements were performed using the VIP^{TM}-200 (NeurOptics, California, US), a battery operated, handheld optical scanner to measure pupil size (i.e. pupil diameter). This instrument can measure pupils in darkness for 2 seconds in no background illumination ("Light off" mode) with an accuracy of 0.1 mm. Under "Variable" mode, pupil diameter can be measured under three different background light conditions: scotopic (light off), low mesopic (0.3 lux), and high mesopic (3 lux) for a total of 10 seconds. For thesis purposes, pupil diameters were measured using the variable mode of the pupillometer and point estimates and standard deviation were obtained and recorded as continuous variables, both in millimetres.

Biothesiometer: T2DM can produce peripheral neuropathy causing pain or sensory loss especially in toes and feet [104]. The damage at vibration perception has been observed in obese individuals even with normal glycemic levels [105]; therefore, it is possible to be used as a screening tool for undiagnosed T2DM. The biothesiometer is a hand-held device used to measure the threshold of vibration perception (appreciation) in human subjects. Although this device can be used for several neurological diseases, it is superior to a tuning fork in accuracy for T2DM neuropathy [106]. A digital biothesiometer model "Vibrometer-VPT" (Diabetik Foot Care India Pvt Ltd, Chennai, India) was used for individuals' assessment. Usually a cut-off of 25 mV is utilised to detect cases of polyneuropathy [104] and a cut-off of 9 mV has been described as abnormal vibration perception threshold [107], but for thesis purposes continuous results were obtained and recorded for analysis.

Variables related to risk scores for undiagnosed T2DM:

Three different risk scores were also assessed and compared to neuropathy assessment tools, the Finnish Diabetes Risk Score (FINDRISC), the Latin American version of the FINDRISC (LA-FINDRISC), and the Peruvian Risk Score.

The FINDRISC is a questionnaire to identify individuals at high risk of developing T2DM. It was created using a prospective cohort study of individuals aged between 35 and 64 years [108]. For easy application, the potential responses of the questions were categorised. The FINDRISC is a blood-free risk score whose original questions included age, body mass index, waist circumference, physical activity, daily consumption of fruits, berries or vegetables, history of anti-hypertensive drug treatment, and history of high blood glucose [109]. However, later studies included family history of T2DM to the model and modified diet patterns and physical activity questions. The English version is available in Appendix E.

Despite the fact that the FINDRISC has been widely used for estimating the risk of developing T2DM within the following ten years, this score has been also evaluated as a tool to identify undiagnosed T2DM, abnormal glucose tolerance, dysglycaemia, and metabolic syndrome [40, 110, 111]. In addition, this tool has been used, adapted and validated in Latin America settings such as Colombia [112, 113], and therefore, it is a valid instrument to be assessed in our population.

On the other hand, the Latin America version of the FINDRISC (LA-FINDRISC) was also included in this evaluation. This questionnaire is very similar to the original FINDRISC, but has been used for detecting cases of impaired glucose regulation and options regarding waist circumference has been adapted for Latin American populations [114]. Thus, cut-offs used for this anthropometric measurement are 94 cm for men and 90 cm for women. The English version is available in Appendix E. Finally, the Peruvian Risk

Score, developed as part of this thesis, was also assessed by using oral glucose tolerance test as the gold standard instead of fasting glucose as originally done [46].

4.6 Demographic and other variables

Sociodemographic variables (age, sex, education level, socioeconomic status, etc.), medical and familial history of T2DM, and lifestyle behaviours (smoking, alcohol consumption, physical activity level, diet patterns, etc.) were taken into account to describe the study population (Definitions are available in Table IV-1, IV-2 and IV-3).

4.7 Data collection methods

After informed consent, participants' information was collected using tablets and measurements were obtained by well-trained clinical personnel. The research team was comprised by five fieldworkers: two staff members were responsible for participant's invitation (i.e. going household by household looking for eligible participant according to selection framework); other two were in charge of data collection and measurements (application of questionnaires and device and anthropometrical assessments), and the latter one person was in charge of blood sampling.

4.7.1 Questionnaires

Participants responded to a face-to-face questionnaire applied by trained health workers using computer-based formats. The Spanish version of the questionnaire is available in Appendix F. An application built using Open Data Kit (ODK: http://opendatakit.lshtm.ac.uk) was utilised using tablets. Using the application, we obtained data about factors potentially associated with T2DM, including sociodemographic variables (age, sex, years of education, socioeconomic variables, etc), behavioural variables (lifestyles comprising smoking habits, alcohol consumption, physical activity levels, diet patterns, clinical symptoms, etc), medical history (T2DM, hypertension, myocardial infarction, and other cardiovascular diseases), and familial medical history focused mainly on glucose metabolism disorder, but also in hypertension and cardiovascular disease.

A modified version of the WHO STEPwise approach to surveillance (WHO STEP) questionnaire for surveillance of chronic non-communicable diseases was used to build the application for data collection [49]. Questions of specific T2DM risk scores (i.e. Peruvian risk score, FINDRISC, among others) were also included.

4.7.2 Clinical assessment

After completing questionnaires, the anthropometric characteristics of participants were also assessed. Measurements of standing height were carried out using a stadiometer and standardised procedures. Weight was assessed using a bio-electrical impedance device (TBF-300A, TANITA Corporation, Tokyo, Japan), as well as waist circumference was assessed in triplicate using standard techniques. Heart rate, systolic and diastolic blood pressure were also evaluated in triplicate using an automatic monitor OMRON HEM-780 (OMRON Healthcare, Illinois, US), previously validated for adult population [50]. Finally, the EZSCAN assessment, pupillometer and biothesiometer evaluation were also performed. Evaluators were blinded to the OGTT results.

EZSCAN assessment:

This evaluation was conducted following the guidelines of the provider [66]. Briefly, the participant was asked to take off his shoes and socks, and then put his/her hands and feet on the electrodes of the EZSCAN. A small electric tension was applied to the surface of hands and feet during about 2 minutes.

After that, percentage of risk is given to indicate the probability of the participant of having/developing T2DM. This result was recorded.

Pupillometry assessment:

Similar to the EZSCAN, evaluation using the pupillometer was carried out using the provider's manual. Both participant eyes were assessed using the "Variable" mode. For this, initially, the lighting of the assessment room was reduced, and at least five minutes were left before starting evaluation (pupil's dark adaptation). Then, the participant's head was aligned with the device to minimize any tilting of the device. The three forms of assessment were used: scotopic (light off), low mesopic (0.3 lux), and high mesopic (3 lux) for a total of 10 – 12 seconds for each eye. All the measurement were recorded in the device and then downloaded to a computer for analysis purposes.

Biothesiometer assessment:

As recommended in the device manual and previous guidelines [115], four sites were tested in each feet: first, third and fifth metatarsal heads and the pulp of the hallux (plantar surface of distal hallux). Measurement in the pulp of the hallux was performed in triplicate to determine the vibration perception threshold as recommended for neuropathy assessment [116]. For evaluation, the participant was asked to be in lying supine position. Then, the stylus of the device was placed over the first point (i.e. first metatarsal head) and the amplitude was increased up to the participant could detect the vibration. The resulting number was the vibration perception threshold. The same procedure was repeated for each point and foot and values were recorded.

4.7.3 Blood sampling

Trained laboratory staff explained procedures for blood sample collection. Participants were asked to provide venous blood sample for OGTT after a minimum of 8 and a maximum of 12 hours of fasting. First blood sampling was obtained at the first moment of the appointment, after verifying fasting period was accomplished. A total of 7.5 ml of venous blood sample was drawn to assess fasting glucose. After that, a load of 75 grams of anhydrous glucose in a volume of 300 ml was used as recommended by the WHO [7]. Two hours after, a new blood sample was obtained to measure glucose levels. In the mid-time, questionnaires and clinical measurements were performed. Thus, we took advantage of the two hours between blood samples to complete questionnaires and to obtain anthropometric and clinical assessments.

Blood testing was carried out by a certified Peruvian laboratory located in Lima (MEDLAB: http://www.medlab.com.pe) using its qualified personnel to do all sampling procedures (blinded to index tests) and to be in charge of transport of samples to the laboratory's facilities. Glucose was measured in serum using a Cobas Modular Platform automated analyzer and reagents supplied by Roche Diagnostics. Quality control for glucose measurements had <1 for the coefficient of variation, a reference range provided by Bio-Rad, an independent assessment company (www.biorad.com).

4.8 Statistical analysis

Following careful data cleaning and consistency checking, descriptive statistics using tabulations and graphical methods was conducted. Analysis was performed using STATA 13.0 for Windows (Stata Corp, College Station, TX, US). Report was conducted using the STARD guidelines [82] and the TRIPOD statement [47] as recommended.

4.8.1 Descriptive analysis

Initially, characteristics of study population were tabulated using proportions in the case of categorical variables, and mean and standard deviation (SD) for continuous variables. After overall participants' description, all cases of known T2DM were excluded from further analyses. Then, the prevalence and 95% confidence interval (95% CI) of undiagnosed T2DM, impaired fasting glucose, impaired glucose tolerance, and dysglycaemia were estimated. In addition, comparison of results obtained using risk scores and neuropathy assessment tools according to OGTT status were also tabulated.

4.8.2 Diagnostic accuracy of scores and neuropathy assessment tools

As measurements of pupillometer and biothesiometer were obtained in each eye and feet, respectively, summary and correlation of results were presented. Correlation was evaluated using Spearman test as non-normal distribution was expected [117]. According to that, the average of each measurement of the pupillometer (i.e. scotopic, low- and high-mesopic) and biothesiometry (first, third and fifth metatarsal head and pulp of the hallux) were estimated and used for further analysis. In the case of the biothesiometer, measurements of the pulp of the hallux were emphasized for analysis as previously recommended [116].

We estimated the diagnostic accuracy of the FINDRISC, the LA-FINDRISC and the Peruvian Risk Score using the c-statistic and the area under the ROC curve. Sensitivity and specificity were also determined as well as optimal empirical cut-off following the method suggested by Youden [54]. Logistic regression was used to evaluate the coefficients of the FINDRISC in Peruvian population and simplify and recalibrate the model. The factors independently associated in the simplified model were each assigned a weighted score, for instance, by dividing the regression coefficients in the final model by the lower coefficient and then rounding them up to the nearest integers as in a previous report [38].

Diagnostic accuracy of the EZSCAN, pupillometer and biothesiometer measurements was also evaluated as with risk scores. Comparison between the performances of risk scores and neuropathy assessment tools was also conducted using the *roccomp* command in STATA. In addition, a combination of potential devices and risk scores using Boolean algebra was also assessed using logistic regression and a two-step approach as previously described [118].

4.9 Ethical considerations

The protocol, informed consent and questionnaires were approved by Ethical Institutional Committee at the Universidad Peruana Cayetano Heredia, Lima, Peru, and London School of Hygiene and Tropical Medicine, London, United Kingdom.

The aims of the study were explained to each participant and informed consent was obtained before commencing any of the activities. Protocol, informed consent forms and questionnaire were reviewed and approved in their Spanish and English versions.

4.10 Institutional support and funding

The present study was carry out in collaboration between CRONICAS Centre of Excellence in Chronic Diseases, at the Universidad Peruana Cayetano Heredia in Peru, and the Department of Epidemiology and Population Health, at London School of Hygiene and Tropical Medicine in United Kingdom. Fieldwork activities were conducted with support of the Centre for Global Health, part of the Universidad Peruana Cayetano Heredia in Tumbes, Peru.

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Figure IV-1: Map of the study setting

Table IV-1: Definition of sociodemographic variables

Variable	Type	Categories	Definition
Age	Continuous		Based on date of birth
Age group	Categorical	<40 years 40 – 49 years 50 – 59 years ≥60 years	
Sex	Categorical	Female Male	Based on self-report
Education level	Categorical	< 7 years 7 – 11 years ≥12 years	Based on the number of years of education obtained at the moment of interview
Socioeconomic status	Categorical	Lowest Middle Highest	Based on household assets possession, and then split in tertiles [119, 120]
Marital status	Categorical	Never married Married Previously married	Self-reported
Currently working	Categorical	No Yes	Self-reported
Monthly personal income	Categorical	Up to 100 PEN 101 – 750 PEN >750 PEN	Self-reported, categorisation based on national minimum wage (750 PEN) during previous 12 months
History of migration	Categorical	No Yes	Self-reported based on response to: “Have you live in Tumbes during all your live?
Health insurance	Categorical	No Yes	Self-reported, based on current affiliation to health insurance

Table IV-2: Definition of lifestyle behaviour variables

Variable	Type	Categories	Definition
History of T2DM in first-degree relatives	Categorical	No Yes	Self-reported
Current smoking	Categorical	Do not smoke Smoke occasionally Smoke daily	Self-reported, based on question of WHO STEPs.
History of smoking	Categorical	Never smoked Smoked before Currently smoke	Self-reported, based on question of WHO STEPs.
Alcohol consumption	Categorical	Never < One per month 1+ times per month	Self-reported, based on frequency of alcohol consumption
Alcohol disorder	Categorical	No Yes	Based on the Alcohol Use Disorder Identification Test (positive if ≥8 points) [121]
Physically active for at least 30 min/day	Categorical	No Yes	Self-reported, based on FINDRISC question
MET score	Categorical	Low Moderate High	Estimates based on the short version of the International Physical Activity Questionnaire (IPAQ)
Watching television	Categorical	<2 hours/day ≥2 but <4 hours/day 4+ hours/day	Self-reported, based on the number of hours watching TV during weekdays and weekends (last week)
Fruits and vegetables	Categorical	< 1 per day	Self-reported, based on FINDRISC question

		≥ 1 per day	
Sweetened juices consumption	Categorical	Up to once/week More than once/week	Self-reported, based on question of Young Lives Study
Soda consumption	Categorical	Up to once/week More than once/week	Self-reported, based on question of Young Lives Study
History of high glucose levels	Categorical	No Yes	Self-reported, based on FINDRISC question

Table IV-3: Definition of anthropometric variables

Variable	Type	Categories	Definition
Weight (in kg.)	Continuous		Measured using a bio-impedance scale
Height (in meters)	Continuous		Measured in standing position using a stadiometer
Body mass index	Continuous		Based on weight and height
Body mass index (categories)	Categorical	Normal Overweight Obese	Based on usual definition of the WHO (<25 kg/m^2, 25 but <30 kg/m^2 and ≥30 kg/m^2)
Waist circumference (in cm.)	Continuous		In triplicate and average of three measures is used
Waist circumference	Categorical	Normal Obese	Based on different definitions for men and women
Systolic blood pressure	Continuous		Based on three measurements after 5 minutes of resting period. Average of two last measures was used for calculations [52].
Diastolic blood pressure	Continuous		
Blood pressure treatment	Categorical	No Yes	Self-reported
Hypertension status	Categorical	No Yes	Based on blood pressure levels, self-reported diagnosis and current treatment.

Chapter V: Feasibility and Pilot Study

5.1 Objectives

The feasibility part of the study aimed to evaluate the logistical and acceptability of using the proposed screening devices in fieldwork. On the other hand, the pilot study was focused on crucial components of the study, including time, costs, staff, and study design before conducting the full-scale project. Secondary objectives comprised the optimisation of practical aspects of the study, including recruitment, paperwork, and data collection.

5.2 Materials, methods and execution

Between August and September 2015, a pilot study was conducted in Tumbes, the area proposed for the main study. A convenience sample of participants with and without T2DM (ratio 1:1), matched by sex and age (±2 years), was planned.

For the pilot purposes, a sample of participants from the CRONICAS Cohort Study, originally enrolled in Tumbes, was re-contacted to be recruited in this study. Details of the CRONICAS Cohort Study have been published elsewhere [48]. Briefly, 3601 participants aged ≥35 years were assessed in 2010-2011 (baseline) and in 2013-2014 (follow-up) to determine the incidence of T2DM among other cardiovascular risk factors. However, for this pilot, only participants from Tumbes were re-contacted.

The reference test was based on two fasting glucose assessments. A positive test was defined as an individual with two fasting plasma glucose measurements (at baseline and follow-up) ≥126 mg/dL or self-reporting anti-diabetic medication, whereas a negative test was defined as an individual with both measurements of glucose <126 mg/dL.

Once individuals were contacted, the objectives of the study were explained and an informed consent was read to confirm participation. A short questionnaire containing information regarding age, sex, lifestyle behaviours, and questions of the Finnish Diabetes Risk Score (FINDRISC questionnaire) was also applied [40]. Anthropometric measurements (height, weight, and waist circumference) as well as blood pressure, after five minutes of resting and in triplicate, were also obtained. Finally, ascertainment with the EZSCAN (sudomotor function) and pupillometry (scotopic, low mesopic and high mesopic diameters) was also undertaken.

For analyses purposes, comparison between individuals with and without T2DM was performed using the Student t-test for independent samples in the case of numerical variables, and Chi-squared test or Fisher exact test for categorical variables. Area under the ROC curve, sensitivity and specificity were also estimated using collected information considering diabetes status as the gold standard. In addition, acceptability of tests (defined as individuals accepting device assessment) was also evaluated.

5.3 Results

A total of 50 individuals with T2DM and 50 controls were enrolled. Mean age among those with T2DM (60.8 years; SD: 10.1) was similar to that of controls (60.7 years; SD: 10.1). Comparisons of demographic characteristics, behaviours, anthropometric measurements and devices results between cases and controls are shown in Table V-1. Of importance, there was significant difference in the low mesopic and high mesopic diameters using pupillometry as well as the FINDRISC score between T2DM cases and controls.

Information regarding the performance of proposed screening devices and risk scores is shown in Table V-2. A better performance was obtained using the FINDRISC score (area under the ROC curve = 0.87), whilst the performance was moderate when using the high mesopic diameter of pupillometry (area under the ROC curve = 0.65).

Finally, of the 82 cases with T2DM re-contacted from the CRONICAS Cohort Study, 20 (24.4%) only accepted questionnaires; thus, only 62 completed assessment including questionnaires and device evaluations, but only 50 could be matched with an appropriate individual without T2DM.

5.4 Utility of results

This pilot study demonstrated that it was possible to perform the study in the selected setting. Measurements were easily obtained and individuals were prone to participate. Additionally, it suggest the possibility to get better performance of the selected devices and scores when applied in the general population and using the OGTT as the gold standard for T2DM diagnosis.

This pilot study suggested that a relatively large research team (4 to 5 health personnel) were needed to conduct the study. In addition, the order of the proposed procedures needed to be pre-specified to appropriately use the two hours gap between OGTT blood samples.

Table V-1: Comparison between individuals with and without T2DM

	With T2DM (n = 50)	Without T2DM (n = 50)	p-value
Sex, female (%)	28 (56.0%)	28 (56.0%)	--
Age, mean (SD)	60.8 (10.1)	60.7 (10.1)	--
Current smoking (%)	4 (8.0%)	4 (8.0%)	0.99
Regular physical activity (%)	23 (46.9%)	32 (64.0%)	0.09
Waist, mean (SD)	100.3 (11.5)	96.8 (11.5)	0.10
Body mass index, mean (SD)	28.5 (5.4)	29.3 (4.9)	0.45
Systolic blood pressure, mean (SD)	129.0 (18.7)	125.5 (20.5)	0.39
Diastolic blood pressure, mean (SD)	76.6 (10.7)	79.0 (11.9)	0.28
Hypertension (%)	28 (56.0%)	18 (36.7%)	0.06
Pupillometry			
Scotopic diameter, mean (SD)	4.09 (0.89)	4.48 (1.04)	0.05
Low mesopic diameter, mean (SD)	4.01 (0.86)	4.47 (1.03)	0.02
High mesopic diameter, mean (SD)	3.86 (0.81)	4.33 (0.98)	0.01
Scores			
FINDRISC score, mean (SD)	18.6 (4.4)	11.6 (4.4)	< 0.001
EZScan			
Sudomotor function, mean (SD)	41.0 (16.4)	36.0 (12.3)	0.09
Insulin resistance, mean (SD)	57.1 (15.9)	51.4 (9.3)	0.03

Table V-2: Comparison of performance between diagnostic tests

Technique	AUC	Sensitivity	Specificity
Pupillometry			
Scotopic diameter	0.60 (0.49 – 0.71)	72.0%	50.0%
Low mesopic diameter	0.63 (0.52 – 0.74)	70.0%	52.1%
High mesopic diameter	0.65 (0.54 – 0.76)	64.0%	62.5%
Scores			
FINDRISC score	0.87 (0.80 – 0.94)	91.3%	64.6%
EZScan			
Sudomotor function	0.59 (0.47 – 0.70)	50.0%	60.0%

AUC = Area under the ROC curve

Chapter VI: Descriptive Results

6.1 Response rates

A total of 2114 individuals were invited to participate in the study. Of them, 486 (22.9%) rejected participation, and 16 (0.8%) women were pregnant and excluded. Of the 1612 (76.3% of the invited) participants enrolled in the study, three did not complete all the procedures; therefore, only 1609 were further analysed. Details of the enrolling procedures are shown in a flowchart in Figure VI-1.

6.2 Characteristics of the study population

6.2.1 Sociodemographic characteristics

Main sociodemographic characteristics are detailed in Table VI-1. There were similar number of males and females (49.7% vs. 50.3%), and the overall age mean was 48.2 (SD: 10.6). Of note, almost a third of participants had less than 7 years of education, 80.4% were married, and 25.7% were migrants.

6.2.2 Lifestyle behaviour characteristics

Among the most important lifestyle behaviours, only 92 (5.7%) reported daily smoking, whereas 121 (7.5%) had alcohol disorder. More than two thirds (68.2%) of the population reported to be physically active (at least 30 min per day); however, using the IPAQ, only 28.2% had high levels of physical activity. Regarding diet patterns, 841 (52.3%) of participants reported consuming at least one fruit or vegetable per day (Table VI-2).

6.2.3 Anthropometric measurements

From the anthropometrical perspective, based on body mass index results, 708 (44.0%) were overweight and 476 (29.6%) were obese. The proportion of individual with obesity using waist circumference and International Diabetes Federation (IDF) definition [122] was 79.4% (n = 1277) and 417 (25.9%) had hypertension. Details of the anthropometric characteristics of study participants are in Table VI-3.

6.3 Prevalence of T2DM and glucose disorders

Based on OGTT results, 176 individuals had T2DM (11.0%; 95%CI: 9.4% - 12.5%), and 105 (59.7%) were aware of their diagnosis. Thus, only 71 (4.7%; 95%CI: 3.7% - 5.8%) individuals had undiagnosed T2DM; whereas this number was 56 (3.5%; 95% CI: 2.6% - 4.5%) when only using fasting glucose.

Regarding glucose disorders, 1159 (77.2%) subjects were normoglycemic, whereas 17 (1.1%; 95% CI: 0.7% - 1.8%) had impaired fasting glucose, and 255 (17.0%; 95% CI: 15.1% - 19.0%) had impaired glucose tolerance. Thus, a total of 343 (22.8%; 95% CI: 20.7% - 25.0%) individuals had dysglycaemia.

When sociodemographic, lifestyle behaviour, and anthropometric characteristics of the study population was evaluated after excluding participants aware of T2DM diagnosis (n = 105), they were very similar to the total sample (Table VI-1, Table VI-2, and Table VI-3), except in the case of self-reported high glucose levels.

6.4 Risk scores and neuropathy assessment tools by OGTT results

Overall, the mean of the three risk scores were greater among those with undiagnosed T2DM than those without T2DM (See Table VI-4). In the case of neuropathy assessment tools, the score using EZSCAN was also greater among those with undiagnosed T2DM ($p < 0.001$). This difference was also present in results of biothesiometer but in the first ($p = 0.001$), third ($p <$

0.001), and fifth ($p < 0.001$) metatarsal head, and not in the pulp of the hallux ($p = 0.05$). There were no differences in the three pupillometer diameters.

6.5 Sex subgroup analysis

There was no difference in the prevalence of undiagnosed T2DM by sex (3.9% among males and 5.6% among females, $p = 0.11$). Behavioural characteristics of the study population according to sex are shown in Appendix G. Of note, there was difference in all the behavioural variables evaluated.

On the other hand, although males had more weight than females (75.8 kg vs. 69.1 kg, $p < 0.001$); females had more obesity using body mass index and waist circumference (See Appendix H). Males had higher levels of systolic and diastolic blood pressure ($p < 0.001$ for both blood pressure levels), but there was no difference in hypertension prevalence ($p = 0.08$).

Finally, when comparing result of risk scores and neuropathy assessment tools according to sex (Appendix I), females had higher total scores in the FINDRISC ($p < 0.001$) and LA-FINDRISC ($p< 0.001$) results, but not in the Peruvian Risk Score ($p = 0.06$). Similarly, values of the EZSCAN and biothesiometer assessments were higher among women than men.

Figure VI-1: Flowchart of study participants

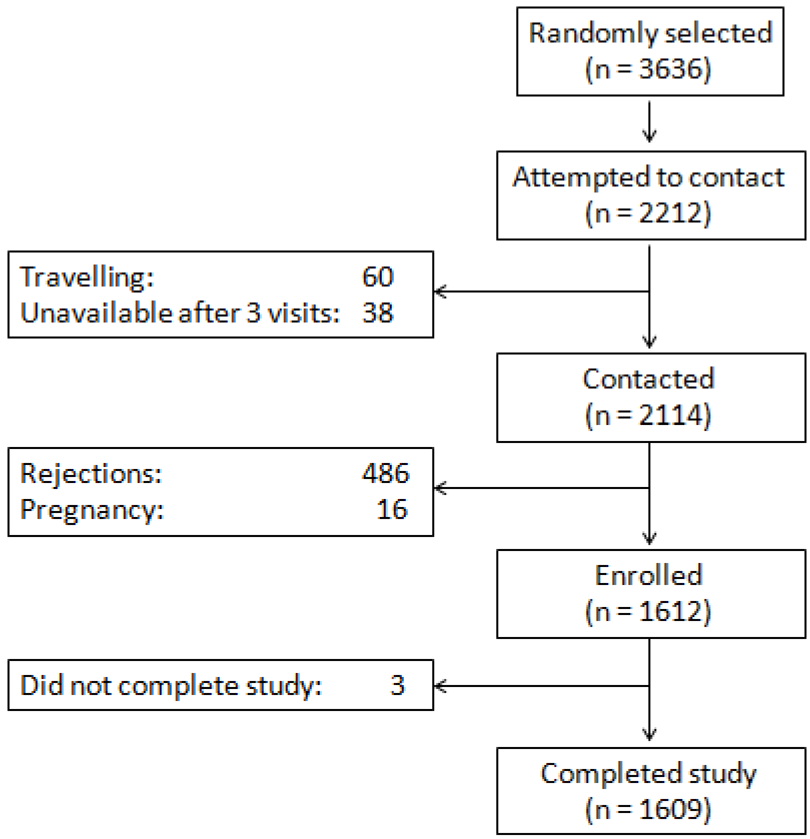

Table VI-1: Sociodemographic characteristics of the total study population and those with OGTT results

		Total population	With OGTT results
		N = 1609	N= 1504
Sociodemographic characteristic		**N (%)**	**N (%)**
Sex	Female	810 (50.3%)	750 (49.9%)
Age	Mean (SD)	48.2 (10.6)	47.6 (10.6)
Education level	< 7 years	519 (32.3%)	466 (31.0%)
	7 – 11 years	749 (46.6%)	708 (47.1%)
	12+ years	341 (21.2%)	330 (21.9%)
Socioeconomic status (tertiles)	Lowest	540 (33.6%)	497 (33.1%)
	Middle	550 (34.2%)	517 (34.4%)
	Highest	519 (32.3%)	490 (32.6%)
Marital status	Never married	163 (10.1%)	156 (10.4%)
	Married	1293 (80.4%)	1211 (80.5%)
	Previously married	153 (9.5%)	137 (9.1%)
Currently working	Yes	1091 (67.8%)	1035 (68.8%)
Monthly personal income	Up to 100 PEN	542 (33.7%)	491 (32.7%)
	101 – 750 PEN	485 (30.2%)	459 (30.5%)
	>750 PEN	581 (36.1%)	553 (36.8%)
History of migration	Yes	413 (25.7%)	385 (25.6%)
Health insurance	Yes	1469 (91.3%)	1368 (91.0%)

Table VI-2: Behavioural characteristics of the total study population and those with OGTT results

Behavioural characteristic		**Total population**	**With OGTT results**
		N = 1609	N= 1504
		N (%)	**N (%)**
T2DM in first-degree relatives	Yes	539 (33.5%)	468 (31.1%)
Smoking			
Current smoking	Do not smoke	1390 (86.4%)	1295 (86.1%)
	Smoke occasionally	127 (7.9%)	123 (8.2%)
	Smoke daily	92 (5.7%)	86 (5.7%)
Self-reported history of smoking	Never smoked	992 (61.7%)	923 (61.4%)
	Smoked before	390 (24.2%)	365 (24.3%)
	Currently smoke	227 (14.1%)	216 (14.4%)
Alcohol use			
Alcohol consumption	Never	686 (42.6%)	618 (41.1%)
	< One per month	770 (47.9%)	736 (48.9%)
	1+ times per month	153 (9.5%)	150 (10.0%)
Alcohol disorder	Yes	121 (7.5%)	121 (8.1%)
Physical activity			
Physically active (≥ 30min/day)	Yes	1098 (68.2%)	1036 (68.9%)
MET score (IPAQ)	Low	605 (37.6%)	550 (36.6%)
	Moderate	551 (34.2%)	519 (34.5%)
	High	453 (28.2%)	435 (28.9%)
Watching television (hours/day)	< 2 hours/day	590 (36.7%)	541 (36.0%)
	≥2 but <4 hours/day	541 (33.6%)	513 (34.1%)
	4+ hours/day	478 (29.7%)	450 (29.9%)
Diet patterns			
Fruits and vegetables	At least one per day	841 (52.3%)	789 (52.5%)
Sweetened juices consumption	≥ Once per week	164 (10.2%)	157 (10.4%)
Soda consumption	≥ Once per week	287 (17.8%)	279 (18.6%)
High glucose levels	Yes	159 (9.9%)	56 (3.7%)

Table VI-3: Anthropometric characteristics of the total study population and those with OGTT results

		Total population	**With OGTT results**
		N = 1609	N= 1504
Anthropometric characteristic		**N (%)**	**N (%)**
Weight (kg)	Mean (SD)	72.3 (13.3)	72.5 (13.3)
Height (m)	Mean (SD)	1.61 (0.1)	1.61 (0.1)
Body mass index (kg/m^2)	Mean (SD)	28.0 (4.6)	28.0 (4.7)
Body mass index (categories)	Normal	425 (26.4%)	399 (26.5%)
	Overweight	708 (44.0%)	655 (43.6%)
	Obese	476 (29.6%)	450 (29.9%)
Waist circumference (cm)	Mean (SD)	93.7 (10.4)	93.6 (10.4)
Waist circumference (IDF categories)	Normal	332 (20.6%)	318 (21.1%)
	Obese	1277 (79.4%)	1186 (78.9%)
Systolic blood pressure (mmHg)	Mean (SD)	119.9 (16.7)	119.5 (16.3)
Diastolic blood pressure (mmHg)	Mean (SD)	79.7 (10.4)	79.5 (10.3)
Blood pressure treatment	Yes	128 (8.0%)	106 (7.1%)
Hypertension status	Yes	417 (25.9%)	370 (24.6%)

Table VI-4: Comparison of results of risk scores and neuropathy assessment tools by undiagnosed T2DM

	Undiagnosed T2DM by OGTT		
	No (N = 1433)	**Yes (N = 71)**	**p-value***
	Mean (SD)	Mean (SD)	
Risk score			
FINDRISC	8.8 (4.2)	11.4 (3.4)	< 0.001
LA-FINDRISC	8.4 (4.4)	11.1 (3.6)	< 0.001
Peruvian Risk Score	1.5 (1.1)	2.0 (1.0)	< 0.001
Neuropathy assessment tool			
EZSCAN	27.0 (10.0)	31.3 (12.9)	< 0.001
Scotopic diameter	4.5 (0.9)	4.4 (0.8)	0.15
Low mesopic diameter	4.5 (0.8)	4.4 (0.8)	0.17
High mesopic diameter	4.3 (0.8)	4.2 (0.8)	0.42
Pulp of the hallux	15.2 (8.5)	17.3 (10.2)	0.05
First metatarsal head	13.6 (7.7)	16.7 (10.2)	0.001
Third metatarsal head	13.5 (7.9)	17 (10.5)	< 0.001
Fifth metatarsal head	13.4 (7.8)	16.7 (10.1)	< 0.001

* P-values were estimated using Student t test for independent samples.

Chapter VII: Diagnostic accuracy of risk scores and neuropathy assessment tools

Using a cross-sectional study to detect cases of undiagnosed (prevalent) T2DM, the diagnostic accuracy of different blood-free risk scores and neuropathy assessment tools was evaluated using the area under the ROC curve and other estimates, including sensitivity and specificity. In this chapter, these results are presented.

7.1 Diagnostic accuracy of risk scores

Performance of the FINDRISC, LA-FINDRISC and the Peruvian Risk Score are detailed in Table VII-1, including area under the ROC curve, empirical cut-off point, sensitivity, specificity, as well as PPV, NPV, likelihood ratio positive and negative, and diagnostic odd ratio (DOR).

7.1.1 FINDRISC performance

The mean score of the FINDRISC in the study population was 8.9 (SD: 4.2) points and values ranged from 0 to 24. When assessing the diagnostic accuracy of the FINDRISC for undiagnosed T2DM, the area under the ROC curve was 0.69 (95% CI: 0.64 – 0.74), with an empirical optimal cut-off point of 11. Using this cut-off, the FINDRISC sensitivity and specificity were 69% (95% CI: 57% - 80%) and 67% (95% CI: 64% - 69%), respectively. When using traditional cut-point of ≥12 as suggested in previous manuscripts [45, 110, 112], sensitivity dropped to 51% (95% CI: 39% - 63%) whereas specificity increased to 74% (95% CI: 72% - 76%).

7.1.2 LA-FINDRISC performance

The mean score of the LA-FINDRISC in the study population was 8.6 (SD: 4.4) points and values ranged from 0 to 24. When assessing the diagnostic accuracy of the LA-FINDRISC for undiagnosed T2DM, the area under the ROC curve was 0.68 (95% CI: 0.63 – 0.74), with an empirical cut-off of 10. Using this cut-point, LA-FINDRISC sensitivity and specificity were 70% (95% CI: 58% – 81%) and 59% (95% CI: 57% - 62%), respectively.

7.1.3 Peruvian Risk Score performance

The mean score of the Peruvian Risk Score in the study population was 1.5 (SD: 1.1) and values ranged from 0 to 4. When assessing the diagnostic accuracy of the Peruvian Risk Score for undiagnosed T2DM, the area under the ROC curve was 0.64 (95% CI: 0.58 – 0.70). When the empirical cut-point of ≥2 was used, the sensitivity of the Peruvian Risk Score was 65% (95% CI: 53% - 76%), whereas the specificity was 54% (95% CI: 51% - 56%).

7.1.4 Simplification of the FINDRISC

Only four variables of the original FINDRISC were independently associated with undiagnosed T2DM in study population: waist circumference (p = 0.005), blood pressure treatment (p = 0.004), history of high blood glucose (p = 0.005), and family history of T2DM (p = 0.02). Coefficients and scores of the simplified version of the FINDRISC are detailed in Table VII-2. The area under the ROC curve of the simplified FINDRISC was 0.71 (95% CI: 0.66 – 0.76), and with an empirical cut-off ≥3, the sensitivity and specificity were 86% (95% CI: 76% - 93%) and 46% (95% CI: 43% - 49%), respectively (Table VII-1).

7.1.5 Comparison of diagnostic accuracy between risk scores

The diagnostic accuracy of the FINDRISC (area under the ROC = 0.69; 95%CI: 0.64 – 0.74) was slightly better than the LA-FINDRISC (area under the ROC = 0.68; 95%CI: 0.63 – 0.74) and the Peruvian Risk Score (area under the ROC = 0.64; 95%CI: 0.58 – 0.70), but results were not significant (p = 0.14). On the other hand, the diagnostic accuracy of the simplified version of the FINDRISC score was similar to the FINDRISC (p = 0.17) and

LA-FINDRISC (p = 0.12), but superior than the Peruvian Risk Score (p = 0.01, Figure VII-1).

7.2 Diagnostic accuracy of neuropathy assessment tools

7.2.1 Performance of the EZSCAN

The mean score of the EZSCAN results was 27.2 (SD: 10.2, range: 8 - 71). When assessing the diagnostic accuracy of the EZSCAN for undiagnosed T2DM, the area under the ROC curve was 0.59 (95% CI: 0.53 – 0.66). When the empirical cut-off of 26 was used, the sensitivity of the EZSCAN was 59% (95% CI: 47% – 71%) and the specificity was 54% (95% CI: 51% – 56%). When the cut-off recommended by the provider was used instead (i.e. 50), the sensitivity dropped to 17% (95% CI: 9% – 28%), whereas the specificity increased to 92% (95% CI: 90% – 93%). These results joined to those obtained using alternative cut-offs as suggested by literature are detailed in Table VII-3.

7.2.2 Performance of the pupillometer

Scotopic diameter:

The mean score of the scotopic diameter in the right eye was 4.5 (SD: 0.9) mm similar to the left eye (mean = 4.5 mm; SD: 0.8), with a Spearman correlation coefficient of 0.85 (p-value < 0.001). The area under the ROC curve was 0.55 (95% CI: 0.49 – 0.62) with an empirical cut-off of 4.2 mm, and a sensitivity of 53% (95% CI: 40% – 65%) and a specificity of 62% (95% CI: 60% – 65%). See details in Table VII-4.

Low-mesopic diameter:

The mean score of the low-mesopic diameter in the right eye was 4.5 (SD: 0.9) mm similar to the left eye (mean = 4.5 mm; SD: 0.9), with a Spearman correlation coefficient of 0.83 (p-value < 0.001). The area under the ROC curve was 0.55 (95% CI: 0.48 – 0.62) with an empirical cut-off of 4.4 mm, and a sensitivity of 54% (95% CI: 42% – 67%) and a specificity of 57% (95% CI: 55% – 60%). Details are shown in Table VII-4.

High-mesopic diameter:

The mean score of the high-mesopic diameter in the right eye was 4.3 (SD: 0.9) mm similar to the left eye (mean = 4.3 mm; SD: 0.8), with a Spearman correlation coefficient of 0.78 (p-value < 0.001). The area under the ROC curve was 0.52 (95% CI: 0.45 – 0.59) with an empirical cut-off of 4.3 mm, and a sensitivity of 53% (95% CI: 40% – 65%) and a specificity of 53% (95% CI: 50% – 55%). Details are shown in Table VII-4.

7.2.3 Performance of the biothesiometer

Using the pulp of the hallux (Table VII-5), the mean of vibration perception threshold in the right and left feet was 15.6 (SD: 9.1) and 14.6 (SD: 9.1), respectively. The Spearman correlation coefficient for both measurements was 0.83 (p-value <0.001). Using the average of right and left vibration perception threshold, the area under the ROC curve for undiagnosed T2DM was 0.55 (95% CI: 0.48 – 0.62) with an empirical cut-off of 20 and a sensitivity of 34% (95% CI: 23% – 46%) and specificity of 78% (95% CI: 76% – 80%).

Vibration perception threshold obtained from metatarsal heads had better diagnostic accuracy than that obtained from the pulp of hallux: areas under the ROC curve were 0.58 (95% CI: 0.51 – 0.65), 0.60 (95% CI: 0.53 – 0.67), and 0.60 (95% CI: 0.52 – 0.67) for the first, third and fifth metatarsal head, respectively. Results are detailed in Table VII-5.

7.2.4 Comparison of diagnostic accuracy between neuropathy assessments tools

Among all the pupillometry indicators, the diagnostic accuracy of the scotopic diameter was similar to the low mesopic diameter (p = 0.41) but slightly better than the high mesopic diameter (p = 0.01, See Figure VII-2).

Similarly, among all the biothesiometer indicators, the diagnostic accuracy of the vibration perception threshold of the first, third and fifth metatarsal heads was better than the pulp of the hallux ($p = 0.03$).

The diagnostic accuracy of the EZSCAN, on the other hand, was better than any of the pupillometer diameters evaluated ($p = 0.03$), but similar to the results of the biothesiometer in the third and fifth metatarsal head ($p = 0.98$).

7.3 Comparison between risk scores and neuropathy assessment tools

The simplified FINDRISC had better diagnostic accuracy when compared to neuropathy devices. Thus, using the c-statistic, the simplified FINDRISC had better performance than the EZSCAN ($p = 0.003$), any pupillometer diameter ($p < 0.001$ for all diameters), and any biothesiometer result (pulp of the hallux, $p < 0.001$; first metatarsal head, $p = 0.005$; third metatarsal head, $p = 0.01$; and fifth metatarsal head, $p = 0.01$). Similarly, the original FINDRISC had better diagnostic accuracy than the EZSCAN ($p = 0.01$), any pupil diameter ($p < 0.001$ for all diameters), and any biothesiometer result ($p = 0.001$). Detailed comparisons between risk scores and neuropathy assessment tools are shown in Table VII-6.

7.4 Combination of risk scores and neuropathy assessment tools

Based on the previous results, specific combinations of two neuropathy assessment tools (EZSCAN and biothesiometer) and the FINDRISC, the LA-FINDRISC and the simplified FINDRISC were performed using Boolean algebra. Both, conjunction (AND) and disjunction (OR) combinations were conducted and evaluated.

7.4.1 Combination of the EZSCAN with blood-free risk scores

EZSCAN and FINDRISC:

A total of 526/1504 (35.0%) participants had a FINDRISC score ≥11 points and were considered at high risk of having undiagnosed T2DM. Among participants with FINDRISC ≥11, 318/525 (60.6%) had an EZSCAN result compatible with undiagnosed T2DM, whereas this number was 390/978 (39.9%) among those who had a FINDRISC <11 points ($p < 0.001$). Subjects with both tests positive (i.e. FINDRISC ≥11 points and EZSCAN ≥26) had more than 5-fold (OR = 5.38; 95% CI: 2.73 – 10.6) increase in the probability of having undiagnosed T2DM compared to those with both tests negative (i.e. FINDRISC <11 points and EZSCAN <26, Table VII-7). If both tests were positive, the sensitivity was 45.1% (95% CI: 33.2% - 57.3%) and the specificity was 80.1% (95% CI: 77.9% - 82.1%). On the other hand, if any of the tests were positive (Table VII-8), the sensitivity and specificity were 83.1% (95% CI: 72.3% - 91.0%) and 40.2% (95% CI: 37.7%- 42.8%), respectively.

EZSCAN and LA-FINDRISC:

A total of 636/1504 (42.3%) participants had a LA-FINDRISC score ≥10 points and were considered at high risk of having undiagnosed T2DM. Among participants with LA-FINDRISC ≥10, 374/635 (58.9%) had an EZSCAN result compatible with undiagnosed T2DM, whereas this number was 334/868 (38.5%) among those who had a LA-FINDRISC <10 points ($p < 0.001$). Subjects with both tests positive (i.e. LA-FINDRISC ≥10 points and EZSCAN ≥26) had more than 4-fold (OR = 4.74; 95% CI: 2.29 – 9.80) increase in the probability of having undiagnosed T2DM compared to those with both tests negative (i.e. LA-FINDRISC <10 points and EZSCAN <26, Table VII-7). If both tests were positive, the sensitivity was 43.7% (95% CI: 31.9% - 56.0%) and the specificity was 76.1% (95% CI: 73.8% - 78.3%). On the other hand, if any of the tests were positive (Table VII-8), the sensitivity and specificity were 85.9% (95% CI: 75.6% - 93.0%) and 36.6% (95% CI: 34.1%- 39.1%), respectively.

EZSCAN and simplified FINDRISC:

A total of 835/1504 (55.5%) participants had a simplified FINDRISC score ≥3 points and were considered at high risk of having undiagnosed T2DM. Among participants with a simplified FINDRISC ≥3, 449/834 (53.8%) had an EZSCAN result compatible with undiagnosed T2DM, whereas this number was 259/669 (38.7%) among those who had a simplified FINDRISC <3 points ($p < 0.001$). Subjects with both tests positive (i.e. simplified FINDRISC ≥3 points and EZSCAN ≥26) had more than 7-fold (OR = 7.27; 95% CI: 2.83 – 18.69) increase in the probability of having undiagnosed T2DM compared to those with both tests negative (i.e. simplified FINDRISC <3 points and EZSCAN <26, Table VII-7). If both tests were positive, the sensitivity was 52.1% (95% CI: 39.9% - 64.1%) and the specificity was 71.3% (95% CI: 68.8% - 73.6%). However, if any of the tests were positive (Table VII-8), the sensitivity and specificity were 93.0% (95% CI: 84.3% - 97.7%) and 28.3% (95% CI: 25.9%- 30.7%), respectively.

7.4.2 Combination of biothesiometer and blood-free risk scores

Biothesiometer and FINDRISC:

Among participants with FINDRISC ≥11, 115/526 (21.9%) had a vibration perception threshold in the third metatarsal head compatible with undiagnosed T2DM, whereas this number was 119/978 (12.2%) among those who had a FINDRISC <11 points ($p < 0.001$). Subjects with both tests positive (i.e. FINDRISC ≥11 points and Biothesiometer in the third metatarsal head ≥21) had more than 8-fold (OR = 8.43; 95% CI: 4.00 – 17.76) increase in the probability of having undiagnosed T2DM compared to those with both tests negative (i.e. FINDRISC <11 points and biothesiometer <21, Table VII-

9). If both tests were positive, the sensitivity was 21.1% (95% CI: 12.3% - 32.4%) and the specificity was 93.0% (95% CI: 91.6% - 94.3%). However, if any of the tests were positive (Table VII-10), the sensitivity and specificity were 78.9% (95% CI: 67.6% - 87.7%) and 58.9% (95% CI: 56.3%- 61.5%), respectively.

Biothesiometer and LA-FINDRISC:

Among participants with LA-FINDRISC ≥10, 133 out of 636 (20.9%) had a vibration perception threshold in the third metatarsal head compatible with undiagnosed T2DM, whereas this number was 101/868 (11.6%) among those who had a LA-FINDRISC <10 points ($p < 0.001$). Subjects with both tests positive (i.e. LA-FINDRISC ≥10 points and Biothesiometer in the third metatarsal head ≥21) had more than 6-fold (OR = 6.85; 95% CI: 3.30 – 14.22) increase in the probability of having undiagnosed T2DM compared to those with both tests negative (i.e. LA-FINDRISC <10 points and biothesiometer <21, Table VII-9). If both tests were positive, the sensitivity was 22.5% (95% CI: 13.5% - 34.0%) and the specificity was 91.8% (95% CI: 90.3% - 93.2%). Nevertheless, if any of the tests were positive (Table VII-10), the sensitivity and specificity were 78.9% (95% CI: 67.6% - 87.7%) and 52.5% (95% CI: 49.9% - 55.1%), respectively.

Biothesiometer and simplified FINDRISC:

Among participants with simplified FINDRISC ≥3, 135/835 (16.2%) had a vibration perception threshold in the third metatarsal compatible with undiagnosed T2DM, whereas this number was 99/669 (14.8%) among those who had a simplified FINDRISC <3 points ($p = 0.47$). Subjects with both tests positive (i.e. simplified FINDRISC ≥3 points and Biothesiometer in the third metatarsal head ≥21) had more than 13-fold (OR = 13.2; 95% CI: 5.4 – 32.0) increase in the probability of having undiagnosed T2DM compared to those with both tests negative (i.e. simplified FINDRISC <3 points and biothesiometer <21, Table VII-9). If both tests were positive, the sensitivity was 26.8% (95% CI: 16.9% - 38.6%) and the specificity was 91.9% (95% CI: 90.4% - 93.3%). Nevertheless, if any of the tests were positive (Table VII-10), the sensitivity and specificity were 90.1% (95% CI: 80.7% - 95.9%) and 39.3% (95% CI: 36.7% - 41.9%), respectively.

Figure VII-1: Comparison of area under the ROC curves between FINDRISC, LA-FINDRISC, Peruvian Risk Score and simplified FINDRISC

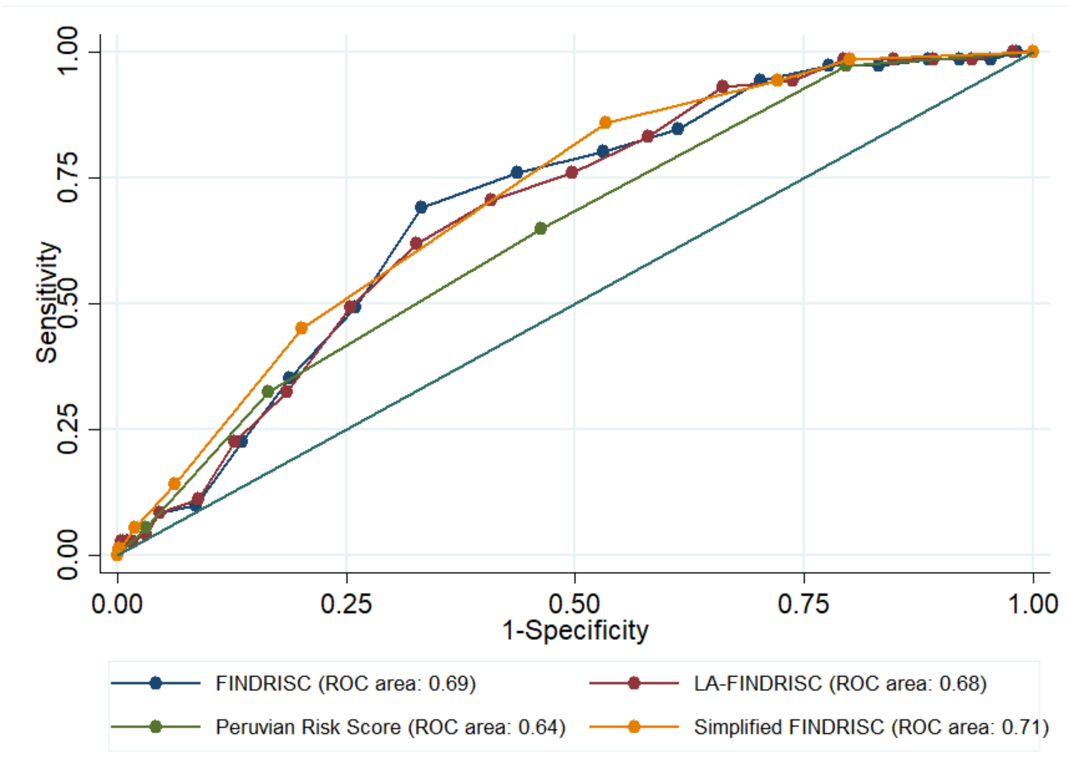

Figure VII-2: Comparison of area under the ROC curves between pupil parameters: scotopic, low-mesopic and high-mesopic diameters

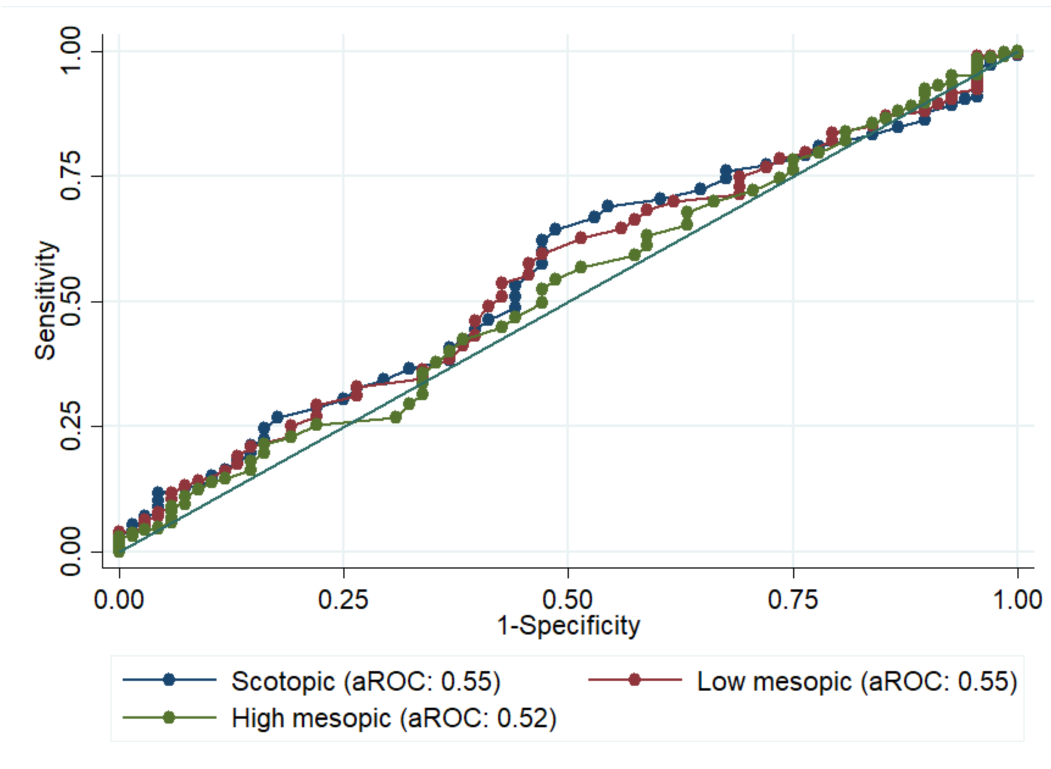

Table VII-1: Diagnostic accuracy of risk scores for undiagnosed T2DM

	FINDRISC	LA-FINDRISC	Peruvian Risk Score	Simplified FINDRISC
	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)
Area under the ROC	0.69 (0.64 – 0.74)	0.68 (0.63 – 0.74)	0.64 (0.58 – 0.70)	0.71 (0.66 – 0.76)
Empirical cut-off	11	10	2	3
Sensitivity	69.0% (56.9% – 79.5%)	70.4% (58.4% – 80.7%)	64.8% (52.5% – 75.8%)	85.9% (75.6% – 93.0%)
Specificity	66.7% (64.2% – 69.2%)	59.1% (56.5% – 61.7%)	53.7% (51.0% – 56.3%)	46.0% (43.4% – 48.6%)
Positive predictive value	9.3% (7.0% – 12.2%)	7.9% (5.9% – 10.2%)	6.5% (4.8% – 8.6%)	7.3% (5.6% – 9.3%)
Negative predictive value	97.7% (96.6% – 98.6%)	97.6% (96.3% – 98.5%)	96.8% (95.4% – 97.9%)	98.5% (97.3% – 99.3%)
Likelihood ratio positive	2.1 (1.8 – 2.5)	1.7 (1.5 – 2.0)	1.4 (1.2 – 1.7)	1.6 (1.4 – 1.8)
Likelihood ratio negative	0.5 (0.3 – 0.7)	0.5 (0.3 – 0.7)	0.7 (0.5 – 0.9)	0.3 (0.2 – 0.5)
Diagnostic odd ratio	4.5 (2.7 – 7.4)	3.4 (2.1 – 5.8)	2.1 (1.3 – 3.5)	5.2 (2.7 – 10.1)

Table VII-2: Coefficients of the simplified FINDRISC for undiagnosed T2DM in Peruvian population

	Bivariable model	Final model*		Score
	OR (95% CI)	Coef. (SE)	OR (95% CI)	
Age (vs. <45 years)				
≥45 and <55 years	1.48 (0.84 – 2.62)			
≥55 and <65 years	1.29 (0.68 – 2.44)			
≥65 years	1.40 (0.52 – 3.74)			
Body mass index (vs. <25 kg/m^2)				
≥25 and <30 kg/m^2	1.58 (0.78 – 3.21)			
≥30 kg/m^2	2.70 (1.34 – 5.43)			
Waist circumference (vs. F<80cm/M<94cm)				
F: ≥80 and <88 cm / M: ≥94 and <102 cm	2.82 (1.17 – 6.83)	0.97 (0.45)	2.63 (1.08 – 6.39)	2 (vs. 0)
F: ≥88 cm / M: ≥102 cm	4.39 (1.97 – 9.83)	1.32 (0.41)	3.75 (1.66 – 8.45)	3 (vs. 0)
Physical activity (vs. no)				
At least 30 min per day	1.14 (0.69 – 1.89)			
Fruits and vegetables intake (vs. no)				
At least once per day	0.96 (0.59 – 1.54)			
Blood pressure medication (vs. no)				
Yes	3.22 (1.71 – 6.10)	0.97 (0.33)	2.64 (1.37 – 5.09)	2 (vs. 0)
History of high blood glucose levels (vs. no)				
Yes	3.74 (1.70 – 8.25)	1.18 (0.42)	3.26 (1.43 – 7.43)	2 (vs. 0)
Family history of T2DM (vs. no)				
Parent, brother, sister or own child	1.87 (1.16 – 3.03)	0.61 (0.25)	1.84 (1.13 – 3.00)	1 (vs. 0)

* The model was created by backward elimination, keeping variables significantly associated with undiagnosed T2DM.

Table VII-3: Diagnostic accuracy of EZSCAN for undiagnosed T2DM: comparison according to different cut-offs

	EZSCAN			
Cut-off	**24**	**26***	**34**	**50**
	Estimate (95% CI)	**Estimate (95% CI)**	**Estimate (95% CI)**	**Estimate (95% CI)**
Sensitivity	78.9% (67.6% – 87.7%)	59.2% (46.8% – 70.7%)	25.4% (15.8% – 37.1%)	16.9% (9.1% – 27.7%)
Specificity	32.0% (29.5% – 34.4%)	53.5% (50.9% – 56.1%)	85.6% (83.7% – 87.4%)	91.7% (90.1% – 93.1%)
Positive predictive value	5.4% (4.1% – 7.0%)	5.9% (4.3% – 8.0%)	8.0% (4.8% – 12.4%)	9.2% (4.8% – 15.5%)
Negative predictive value	96.8% (94.8% – 98.2%)	96.3% (94.8% – 97.5%)	95.8% (94.6% – 96.9%)	95.7% (94.5% – 96.7%)
Likelihood ratio positive	1.2 (1.0 – 1.3)	1.3 (1.0 – 1.6)	1.8 (1.2 – 2.7)	2.0 (1.2 – 3.5)
Likelihood ratio negative	0.7 (0.4 – 1.0)	0.8 (0.6 – 1.0)	0.9 (0.8 – 1.0)	0.9 (0.8 – 1.0)
Diagnostic odd ratio	1.8 (1.0 – 3.1)	1.7 (1.0 – 2.7)	2.0 (1.2 – 3.5)	2.2 (1.2 – 4.3)

* Best cut-off according to Youden's method.

Table VII-4: Diagnostic accuracy of pupil diameters for undiagnosed T2DM

	Scotopic diameter	Low-mesopic diameter	High-mesopic diameter
	Estimate (95% CI)	**Estimate (95% CI)**	**Estimate (95% CI)**
Area under the ROC curve	0.55 (0.49 – 0.62)	0.55 (0.48 – 0.62)	0.52 (0.45 – 0.59)
Empirical cut-off	4.2	4.4	4.3
Sensitivity	52.9% (40.4% – 65.2%)	54.4% (41.9% – 66.5%)	52.9% (40.4% – 65.2%)
Specificity	62.2% (59.6% – 64.7%)	57.4% (54.8% – 60.0%)	52.6% (49.9% – 55.2%)
Positive predictive value	6.3% (4.5% – 8.6%)	5.8% (4.1% – 7.9%)	5.1% (3.6% – 7.0%)
Negative predictive value	96.5% (95.1% – 97.6%)	96.3% (94.8% – 97.5%)	95.9% (94.2% – 97.2%)
Likelihood ratio positive	1.4 (1.1 – 1.8)	1.3 (1.0 – 1.6)	1.1 (0.9 – 1.4)
Likelihood ratio negative	0.8 (0.6 – 1.0)	0.8 (0.6 – 1.0)	0.9 (0.7 – 1.2)
Diagnostic odd ratio	1.9 (1.1 – 3.0)	1.6 (1.0 – 2.6)	1.3 (0.8 – 2.0)

Table VII-5: Diagnostic accuracy of biothesiometer indicators for undiagnosed T2DM

	Pulp of hallux	First metatarsal head	Third metatarsal head	Fifth metatarsal head
	Estimate (95% CI)	**Estimate (95% CI)**	**Estimate (95% CI)**	**Estimate (95% CI)**
Area under the ROC curve	0.55 (0.48 – 0.62)	0.58 (0.51 – 0.65)	0.60 (0.53 – 0.67)	0.60 (0.52 – 0.67)
Empirical cut-off	20	21	21	21
Sensitivity	33.8% (23.0% – 46.0%)	31.0% (20.5% – 43.1%)	31.0% (20.5% – 43.1%)	32.4% (21.8% – 44.5%)
Specificity	78.1% (75.8% – 80.2%)	85.7% (83.8% – 87.5%)	85.2% (83.2% – 87.0%)	86.1% (84.2% – 87.8%)
Positive predictive value	7.1% (4.6% – 10.4%)	9.7% (6.2% – 14.4%)	9.4% (6.0% – 13.9%)	10.4% (6.7% – 15.1%)
Negative predictive value	96.0% (94.7% – 97.0%)	96.2% (95.0% – 97.1%)	96.1% (94.9% – 97.1%)	96.3% (95.1% – 97.2%)
Likelihood ratio positive	1.5 (1.1 – 2.2)	2.2 (1.5 – 3.2)	2.1 (1.5 – 3.0)	2.3 (1.6 – 3.3)
Likelihood ratio negative	0.8 (0.7 – 1.0)	0.8 (0.7 – 0.9)	0.8 (0.7 – 0.9)	0.8 (0.7 – 0.9)
Diagnostic odd ratio	1.8 (1.1 – 3.0)	2.7 (1.6 – 4.5)	2.6 (1.5 – 4.3)	3.0 (1.8 – 5.0)

Table VII-6: Comparisons between risk scores and neuropathy assessment tools for undiagnosed T2DM

Neuropathy assessment tools		**Blood free risk scores**			
		FINDRISC	**LA-FINDRISC**	**Peruvian Risk Score**	**Simplified FINDRISC**
	aROC*	0.69 (0.64 – 0.74)	0.68 (0.63 – 0.74)	0.64 (0.58 – 0.70)	0.71 (0.66 – 0.76)
EZSCAN	0.59 (0.53 – 0.66)	0.01	0.02	0.19	0.003
Scotopic diameter	0.55 (0.49 – 0.62)	0.002	0.006	0.16	< 0.001
Low mesopic diameter	0.55 (0.48 – 0.62)	< 0.001	0.001	0.07	0.001
High mesopic diameter	0.52 (0.45 – 0.59)	< 0.001	0.001	0.01	< 0.001
Pulp of hallux	0.55 (0.48 – 0.62)	0.002	0.004	0.04	< 0.001
First metatarsal head	0.58 (0.51 – 0.65)	0.01	0.03	0.16	0.005
Third metatarsal head	0.60 (0.53 – 0.67)	0.03	0.05	0.28	0.01
Fifth metatarsal head	0.60 (0.52 – 0.67)	0.03	0.05	0.27	0.01

P-values are shown to detail differences between risk scores and neuropathy assessment devices
* aROC = Area under the ROC curve

Table VII-7: Association between the combination of EZSCAN and blood-free risk scores for undiagnosed T2DM

	OR (95% CI)
FINDRISC and EZSCAN	
FINDRISC <11 points, EZSCAN <26	1 (Reference)
FINDRISC <11 points, EZSCAN ≥26	1.26 (0.54 – 2.95)
FINDRISC ≥11 points, EZSCAN <26	4.29 (2.01 – 9.14)
FINDRISC ≥11 points, EZSCAN ≥26	5.38 (2.73 – 10.60)
LA-FINDRISC and EZSCAN	
LA-FINDRISC <10 points, EZSCAN <26	1 (Reference)
LA-FINDRISC <10 points, EZSCAN ≥26	1.78 (0.75 – 4.24)
LA-FINDRISC ≥10 points, EZSCAN <26	4.11 (1.88 – 8.96)
LA-FINDRISC ≥10 points, EZSCAN ≥26	4.74 (2.29 – 9.80)
Simplified FINDRISC and EZSCAN	
Simplified FINDRISC <3 points, EZSCAN <26	1 (Reference)
Simplified FINDRISC <3 points, EZSCAN ≥26	1.59 (0.46 – 5.55)
Simplified FINDRISC ≥3 points, EZSCAN <26	5.37 (2.03 – 14.23)
Simplified FINDRISC ≥3 points, EZSCAN ≥26	7.27 (2.83 – 18.69)

Table VII-8: Combination of risk scores and EZSCAN: Diagnostic accuracy for undiagnosed T2DM

	EZSCAN combined with...		
	FINDRISC	**LA-FINDRISC**	**Simplified FINDRISC**
	Estimate (95% CI)	**Estimate (95% CI)**	**Estimate (95% CI)**
Sensitivity	83.1% (72.3%–91.0%)	85.9% (75.6%–93.0%)	93.0% (84.3%–97.7%)
Specificity	40.2% (37.7%–42.8%)	36.6% (34.1%–39.1%)	28.3% (25.9%–30.7%)
Positive predictive value	6.5% (5.0%–8.3%)	6.3% (4.9%–8.0%)	5.2% (2.1%–12.6%)
Negative predictive value	98.0% (96.5%–98.9%)	98.1% (96.6%–99.1%)	98.8% (97.2%–99.6%)
Likelihood ratio positive	1.4 (1.2 – 1.6)	1.4 (1.2 – 1.5)	1.3 (1.2 – 1.4)
Likelihood ratio negative	0.4 (0.3 – 0.7)	0.4 (0.2 – 0.7)	0.2 (0.1 – 0.6)
Diagnostic odd ratio	3.3 (1.8 – 6.2)	3.5 (1.8 – 6.8)	5.2 (2.1 – 12.6)

Table VII-9: Association between the combination of biothesiometer and blood-free risk scores for undiagnosed T2DM

	OR (95% CI)
FINDRISC and Biothesiometer*	
FINDRISC <11 points, Biothesiometer <21	1 (Reference)
FINDRISC <11 points, Biothesiometer ≥21	3.51 (1.40 – 8.80)
FINDRISC ≥11 points, Biothesiometer <21	5.08 (2.73 – 9.44)
FINDRISC ≥11 points, Biothesiometer ≥21	8.43 (4.00 – 17.76)
LA-FINDRISC and Biothesiometer*	
LA-FINDRISC <10 points, Biothesiometer <21	1 (Reference)
LA-FINDRISC <10 points, Biothesiometer ≥21	3.16 (1.20 – 8.35)
LA-FINDRISC ≥10 points, Biothesiometer <21	3.64 (1.96 – 6.75)
LA-FINDRISC ≥10 points, Biothesiometer ≥21	6.85 (3.30 – 14.22)
Simplified FINDRISC and Biothesiometer*	
Simplified FINDRISC <3 points, Biothesiometer <21	1 (Reference)
Simplified FINDRISC <3 points, Biothesiometer ≥21	2.51 (0.64 – 9.87)
Simplified FINDRISC ≥3 points, Biothesiometer <21	5.13 (2.29 – 11.51)
Simplified FINDRISC ≥3 points, Biothesiometer ≥21	13.15 (5.40 – 32.00)

* The third metatarsal head was used for analyses

Table VII-10: Combination of risk scores and biothesiometer: Diagnostic accuracy for undiagnosed T2DM

	Biothesiometer* combined with...		
	FINDRISC	**LA-FINDRISC**	**Simplified FINDRISC**
	Estimate (95% CI)	**Estimate (95% CI)**	**Estimate (95% CI)**
Sensitivity	78.9% (67.6%–87.7%)	78.9% (67.6%–87.7)	90.1% (80.7%–95.9%)
Specificity	58.9% (56.3%–61.5%)	52.5% (49.9%–55.1%)	39.3% (36.7%–41.9%)
Positive predictive value	8.7% (6.6%–11.1%)	7.6% (5.8%–9.8%)	5.9% (2.7%–12.8%)
Negative predictive value	98.3% (97.1%–99.0%)	98.0% (96.8%–98.9%)	98.8% (97.5%–99.5%)
Likelihood ratio positive	1.9 (1.7 – 2.2)	1.7 (1.5 – 1.9)	1.5 (1.4 – 1.6)
Likelihood ratio negative	0.4 (0.2 – 0.6)	0.4 (0.3 – 0.6)	0.3 (0.1 – 0.5)
Diagnostic odd ratio	5.4 (3.0 – 9.5)	4.1 (2.3 – 7.3)	5.9 (2.7 – 12.8)

* The third metatarsal head was used for analyses

Chapter VIII: Discussion of Findings

There is a need to identify individuals with undiagnosed T2DM. Despite international recommendations, the application of blood markers, such as fasting glucose, oral glucose tolerance test or glycated haemoglobin, will not always be affordable in low- and middle-income countries such as Peru, as laboratory and human resources are needed to obtain appropriate results. Moreover, even if blood markers are used, stepwise approaches (using risk score or non-invasive methods including neuropathy assessment tools) could be a pragmatic and efficient way to reduce the number of invasive test needed for detecting T2DM cases.

Thus, the inclusion of alternative methods such as blood-free risk scores needs to be evaluated in these contexts. In addition, there are many devices used to assess autonomic dysfunction, although most require well-trained staff. There are, however, some devices (EZSCAN, pupillometer and biothesiometer) that can be used to evaluate neuropathy dysfunction and that easy to use, are not time demanding, and do not require fasting. These devices may have a role in T2DM screening.

A cross-sectional population-based study was conducted to assess the diagnostic accuracy of existing blood-free risk scores, mainly focused on the FINDRISC and the Peruvian Risk Score, and neuropathy assessment tools, mainly the EZSCAN, pupillometer and biothesiometer, for undiagnosed T2DM at the population level. To accomplish this, a random sample of more than 1500 participants was assessed using tablet-based questionnaires, anthropometric markers and blood samples to respond our hypotheses.

In this chapter, the discussion of the findings overall are performed in the context of previous existing studies.

8.1 Main findings

This study assessed the diagnostic accuracy of four blood-free risk scores (FINDRISC, LA-FINDRISC, the Peruvian Risk Score, and the simplified FINDRISC) and three different neuropathy assessment tools (EZSCAN, pupillometer and biothesiometer) for detecting cases of undiagnosed T2DM. Although we anticipated a sensitivity of at least 75% compared to OGTT as the gold standard, none of the tools or risk scores reached such sensitivity. A basic Boolean algebra combination using disjunction terms (OR), instead of conjunction terms (AND), of blood-free risk score models and neuropathy assessment tools was necessary in order to reach the proposed 75% sensitivity.

8.2 Diagnostic accuracy of risk scores for undiagnosed T2DM

8.2.1 Summary

Our findings demonstrated that the diagnostic accuracy of the FINDRISC, LA-FINDRISC and the Peruvian Risk Score for undiagnosed T2DM were similar. However, a simplified version of the FINDRISC, including waist circumference, blood pressure treatment, history of high blood glucose levels, and family history of T2DM, could perform similar to the FINDRISC and LA-FINDRISC, but better than the Peruvian Risk Score. These four variables are easy to obtain in clinical practice and thus, can be implementable for detecting undiagnosed T2DM at the population level.

8.2.2 Comparison with previous studies

There are many risk scores created for detecting cases of undiagnosed T2DM worldwide, though many of them are for Caucasian [37-39] and Asian populations [56, 57, 123]. These risk scores can be blood-free models or can contain blood results such as glycated haemoglobin, fasting glucose or lipid markers [26, 36, 124]. However, the interest of this thesis is focused on blood-free risk scores for undiagnosed T2DM.

Among the existing blood-free risk models, the FINDRISC is a well-known score created initially for incident T2DM cases, but currently is used for T2DM screening [40]. However, previous experience has established that a risk score needs to be adapted, validated, or calibrated in the population where this is planned to be applied as prevalence and distribution of outcomes and risk factors are not similar between settings [125].

The FINDRISC had a moderate performance for detecting cases of undiagnosed T2DM in Peruvian population. Our results were similar to previous studies in Latin America [45, 113], although the diagnostic accuracy was lower than in Asian [111] or European [40, 126] populations. Moreover, according to our logistic regression model, the original FINDRISC can be simplified to only four variables to slightly improve the diagnostic accuracy but also facilitate its application and implementation.

Of note, and in contrast with many other risk models [37, 38, 40, 41, 127-131], age was not an independent factor associated with undiagnosed T2DM in our simplified FINDRISC. Age has been described as a risk factor related to type 2 diabetes mellitus with an increasing age associated with increasing probability of T2DM. However, only few risk scores has not included age in the final model [132-134]. Apparently, the probability of having undiagnosed T2DM can be considered similar between age groups in our population despite our study included a wide range of participant ages (i.e. from 30 to 69 years). Moreover, the Peruvian Risk Score included age in the final model [46]. A post-hoc analysis of our data showed that in a crude model, an increase of one year in age was associated with an increase of 2% in the probability of having undiagnosed T2DM ($p < 0.05$); but, in the multivariable model, this estimate dropped to 1% and was not significant ($p = 0.39$). This finding can be important as apparently our results suggest that all individuals over 30 years should be screened, at least with a risk score, for T2DM.

8.3 Neuropathy assessment tools for undiagnosed T2DM

Three different neuropathy assessment tools (EZSCAN, pupillometer and biothesiometer) were assessed for detecting cases of undiagnosed T2DM. In the following lines, a discussion about the diagnostic accuracy of each of the devices as well as a comparison with existing literature is performed.

8.3.1 Diagnostic accuracy of EZSCAN for undiagnosed T2DM

Summary

According to our study findings, the diagnostic accuracy of the EZSCAN was not as good in our population as expected. The trade-off between sensitivity and specificity using the Youden index did not reach the hypothesised sensitivity to detect cases of undiagnosed T2DM. Despite of this, the EZSCAN had, among the assessed neuropathy assessment tools, one of the best diagnostic accuracy for detecting cases of undiagnosed T2DM.

Comparison with previous studies

The EZSCAN has been proposed as an appropriate tool to detect individuals at risk of T2DM [100] and also cases of pre-diabetes and dysglycaemia [75, 77, 135]. A relatively recent systematic review and meta-analysis [63] reported an EZSCAN sensitivity of 72%, but analyses only included information of studies from China, India and Mexico, countries with a higher prevalence of T2DM compared to Peru. Moreover, two of the four studies included in the meta-analysis reported a sensitivity ≥75% [75, 77], but as there is always a trade-off between sensitivity and specificity, it seems likely that these two latter studies sacrificed specificity to detect more cases.

Only one study from the systematic review was conducted using a population-based sample [75], whereas the other three studies enrolled individuals from routine health checkups. Besides, one study used fasting glucose as the gold standard instead of OGTT [78]. The update search of the systematic review found another study [83], but also with selection bias as participants were recruited for health check-ups, and because of that, results did not change markedly.

In post-hoc analysis (HS-ROC), when our study findings were included in the meta-analysis (i.e. a total of 6 studies were analysed instead of the original 4 and the updated version of 5 studies), summary sensitivity and specificity were 70.8% (95%CI: 62.5% – 78.0%) and 61.6% (95%CI: 49.4% – 72.5%), respectively. In addition, when this meta-analysis was conducted excluding the only study using fasting glucose [78], the sensitivity and specificity were 70.2% (95% CI: 60.1% - 78.6%) and 59.7% (95% CI: 45.5% - 72.4%), respectively. These post-hoc results suggest than the diagnostic accuracy of the EZSCAN is not the same when evaluated using a population level approach compared to clinical settings.

8.3.2 Diagnostic accuracy of pupillometry for undiagnosed T2DM

Summary

Three different pupil diameters, part of the response of pupil to light, were assessed in this study. Our findings indicate that none of the pupil measurements was different between individuals with and without T2DM; and for instance, none of them had adequate diagnostic accuracy for undiagnosed T2DM.

Comparison with previous studies

There are many studies reporting differences in several pupil measurements between individual with and without T2DM [136-141]. There are also many techniques and devices utilised to determine pupil diameters, including the use of pupillometer [85, 86, 89-91, 93, 140], pupillography [84, 87, 137], infrared light reflex technique [88, 136, 139], photographic camera [141, 142], and pupil gauge [138]. Thus, there is also many pupil measurements reported, depending basically upon the utilization of static or dynamic pupillometry.

The measurement of the pupil diameter is one of the most common parameters used to compare individuals with and without T2DM [85, 87, 93, 138] and has been used to predict the risk of diabetic neuropathy and cardiovascular autonomic neuropathy [85, 86, 88, 91, 141] more than for undiagnosed T2DM screening. Previous studies have reported obvious differences in pupil parameters between T2DM and non-T2DM subjects. These differences were not found in this study; probably because more severe cases (i.e. those with longer time of disease) were excluded and only undiagnosed T2DM (i.e. newly-diagnosed or screen-detected) cases were analysed. In the same line, there were significant differences in our three pupil diameters in the pilot study but not in our population study. This finding might be because T2DM cases in the pilot study were those who had confirmed diagnosis of type 2 diabetes mellitus (two glycaemia measures $\geq$ 126 mg/dL over a period of 3 years).

Similarly, *Kuroda et al* [87] reported no differences in pupil diameter and pupil area between controls without T2DM and borderline T2DM, but these both categories were statistically different from overt non-insulin dependent T2DM cases. In this latter study, a borderline T2DM case was defined "by the results of a 50 grams oral glucose tolerance test and the criteria set out by the Japanese Diabetic Society" [87], a category almost comparable to the definition of newly-diagnosed T2DM. Thus, these findings suggest that, although there are some pupil changes due to glucose metabolism disorder from the beginning of the disease, these changes may be so small they cannot be detected by a static pupillometer.

To my knowledge, only one study reported the use of pupil indicators, utilizing dynamic pupillometry, as a screening tool of T2DM. *Lerner et al* found that diagnostic accuracy of several pupil measurements were fair enough for T2DM screening (area under the ROC curve ≥ 0.60), including pupil diameter, amplitude of pupil reaction, and constriction ratio [20]. However, cases with T2DM were not undiagnosed but instead a combination of patients with T2DM from healthcare facilities and general population defined using OGTT. This definition could have an impact on the differences reported as only 5% of the general population had more than 10 years of disease compared to 46% in the hospital-based group. What is more, the control group was defined in a community setting, which can increase the probability of finding differences between T2DM and non-T2DM groups.

8.3.3 Diagnostic accuracy of biothesiometer for undiagnosed T2DM

Summary

As evaluated by the c-statistic as well as sensitivity, none of the vibration perception thresholds in the four foot areas was good enough to detect cases of undiagnosed T2DM. Thus, the biothesiometer alone could not be considered as an adequate screening tool; however, given their specificity, they could be used to discard T2DM.

Comparison with other studies

Biothesiometer is a device routinely used for peripheral neuropathy screening in patients with T2DM [96, 115, 116]. Its performance for detecting cases of large nerve fibre dysfunction in lower extremities has been previously described as superior to the tuning fork and the 10-g monofilament [143]; however, to our knowledge, it has not been used for detecting cases of undiagnosed T2DM.

Some studies have reported the presence of peripheral neuropathy and abnormal vibration perception threshold among cases of undiagnosed T2DM. For example, in a study conducted in India, about 30% of participants with undiagnosed T2DM had peripheral neuropathy and almost 45% had abnormal vibration perception threshold [107]. However, in this latter manuscript, a newly diagnosed T2DM case was defined as those individuals with a diagnosis of <6 months at the moment of the clinical evaluation. In addition, a cut-off of >9 mV in any of the first toes was used to define a participant as having abnormal vibration perception threshold. On the other hand, another study reported the presence of peripheral neuropathy among newly-diagnosed T2DM cases being lower than 10% [144], but only using monofilament and tuning fork for diagnosis.

Studies suggest that peripheral neuropathy in T2DM is multifactorial, but the exact causes are not completely understood [104]. Currently, there are enough proofs to believe that oxidative and inflammatory stress can play an important role in the nerve cells damage, even when an individual has metabolic syndrome [145, 146]. Thus, a cascade of reactions with the subsequent nerve fibre loss is present even before having T2DM diagnosis [147]; but, apparently, this damage cannot be detected by a neuropathy assessment tool as the biothesiometer.

8.4 Combination of risk scores and neuropathy assessment tools

8.4.1 Summary

Based on our findings, the combination of the simplified FINDRISC with the EZSCAN or biothesiometer improved sensitivity over 90%, without affecting the area under the ROC curve, but with reduced specificity. However, the combination of the original FINDRISC and biothesiometer had a sensitivity ≥75% as originally proposed, and the specificity was close to 60%.

8.4.2 Comparison with previous studies

According to literature, only one study assessed the potential benefit of adding a neuropathy assessment tool to a risk score. In a study conducted in China, using a population-based sample, a risk model was created using age, body mass index, family history of T2DM, history of cardiovascular disease, systolic blood pressure, diastolic blood pressure, high-density lipoprotein (HDL) cholesterol, triglycerides, and women who delivered a giant baby or who were diagnosed with gestational diabetes mellitus, and then combined with the EZSCAN to detect cases of undiagnosed T2DM [75]. The area under the ROC curve for the risk model without EZSCAN was 0.68 (95% CI: 0.66 – 0.69), but including EZCAN only slightly improved (0.70; 95% CI: 0.69 – 0.72), reaching similar values as our proposed EZSCAN and FINDRISC combination.

A different report described a two-step approach for incident T2DM cases [118]. The authors used the San Antonio Diabetes Prediction Model as the initial screening, and then used the 1-hour plasma glucose after 75-grams OGTT. With this combination they demonstrated an increasing trend in the risk of developing T2DM when evaluated in two different cohort studies, with a sensitivity of 78% and a specificity of 77%. Similar to our findings, our model using a combination of the FINDRISC and the biothesiometer showed an increased sensitivity for detecting cases of undiagnosed T2DM, with a relatively acceptable specificity (Table VII-6).

All these results suggest that, similar to blood markers (FG, 2-hour glucose tolerance test or HbA1c), T2DM cannot be easily detected by using one risk score model or one neuropathy assessment tool, but instead different tests seems to detect different T2DM cases. Recently, a paper reported that it was possible to create different subgroups of T2DM cases according to disease progression [148]. For instance, results provided by neuropathy assessment tools can be linked to the risk of complications in these subgroups.

According to the National Institute for Health and Clinical Excellence (NICE) guidelines [149], the use of non-invasive screening tools is recommended to identifying individuals at high risk of T2DM, and these screening tools can be undertaken as a self-assessment or as opportunistic assessment in clinical practice. Besides, the utilization of a multi-step approach may increase the response rate to the invitation to T2DM screening, reducing the number of tests needed for a definite diagnosis [27]. Thus, the combination of the FINDRISC and biothesiometer might help to identify individuals with T2DM.

8.5 Prevalence of T2DM and undiagnosed T2DM

8.5.1 Summary

A T2DM prevalence of 11% of was found in this study. Out of all the cases with T2DM, 60% of individuals were aware of their diagnosis, and hence, 40% had undiagnosed T2DM.

8.5.2 Comparison with previous studies

The International Diabetes Federation estimates that between 38% and 69% of individuals with T2DM are unaware of their diagnosis [5]. Previous studies, conducted in low- and middle-income countries, have reported similar results [150-152]. For example, *Shen et al* reported a prevalence awareness of 80% in South Asia and Latin America, whereas Africa had the lowest awareness (66%) [151]. Our results in a semi-urban setting are lower than those reported in Africa or those in urban settings as described in Argentina and Chile [150]. For example, a previous review reported that unawareness in rural and semiurban areas can reach values close to 100% [153]; pointing out the need to have appropriate strategies to reduce the burden of T2DM unawareness.

Chapter IX: Relevance of the Findings

In this final chapter, a discussion of the strengths and limitations of this study as well as the relevance and implications for research are discussed. In addition, further research steps and conclusions are also presented.

9.1 Strengths of the study

- This study is the first study to my knowledge to use oral glucose tolerance test (OGTT) at the population level in Peru. The use of OGTT is time consuming and expensive in resource-constrained settings but it was needed to avoid verification bias [154]. The use of OGTT demanded appropriate logistics to guarantee adequate results.
- A random sample of participants taken from general population and using the most updated census in the area was enrolled for this study. In addition, a sex-stratified sample was used to guarantee an appropriate and comparable number of individuals from both sexes.
- Conduction of a pilot study to optimize the fieldwork activities for the larger study. Thus, the pilot study provided information to organize and structure our research activities using the two hours required by the OGTT.
- Good response rates: more than 75% of invited participants were enrolled in the study. In addition, among the participants enrolled, almost all, but three, completed all the study procedures, including questionnaires, measurements and blood sampling.
- High quality data generated using open-source software (ODK) and tablet-based formats which guaranteed low rates of missing values and inconsistencies. In addition, the evaluation of three different neuropathy

assessment tools and well-established risk score models was conducted using standardised procedures.

- Analysis conducted under standard international recommendations and guidelines: Standard for Reporting of Diagnostic Accuracy Studies (STARD) and the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD).

9.2 Limitations of the study

- Despite of the random sampling used, selection bias may have arisen as only one Peruvian region, with the highest prevalence of obesity and T2DM, was selected and evaluated for this study. As pointed out above, prevalence of the outcome as well as distribution of risk factors are not similar between settings [125], and can play an important role in the validation and especially the calibration of risk scores. In addition, as 23% of contacted individuals rejected participation in the study, participation bias may be another issue limiting generalisation of our findings. However, our results, especially those related with prevalence and awareness, are very similar to previous studies in the region [17, 151]. Although these two biases can have an impact on the generalization of results, our study adds value to previous findings by using OGTT as the gold standard for undiagnosed T2DM.

- Some desirability and recall bias may be present especially in questions about lifestyle behaviours. For example, more than two thirds participants reported being physically active (i.e. exercise for at least 30 minutes per day) and almost half of them reported consuming fruits and vegetables at least once a day. These questions were included as the original FINDRISC, not using a validated scale. When compared to the International Physical

Activity Questionnaire (IPAQ), only a third of the population has high levels of physical activity compared to two thirds of the FINDRISC item.

- Misclassification may be another problem in the study as only one test (OGTT) was carried out instead of the two tests (OGTT, fasting glucose or HbA1c) required for confirmation as recommended in guidelines [7]. Given the high number of participants enrolled in this study, as in other reports with huge sample size [155-157], it is very difficult to conduct population-based studies using two tests for confirmation of T2DM.
- Although the sample was originally stratified by sex, sample size was not enough to guarantee the evaluation of risk scores and neuropathy assessment tools by sex. As prevalence of risk factors was markedly different between sexes, further studies are needed to assess the diagnostic performance of the scores and devices used in the present study.
- Our regression models were created based on the idea of risk stratification instead of individualization [134]; therefore, some variables (BMI and waist circumference, but also results of neuropathy assessment tools) were categorised instead of being kept as numerical in the risk score. Our original idea was to develop a simple score for detecting cases of undiagnosed T2DM as in the original FINDRISC instead of a complicate algorithm; thus, our score or combination of scores can easily be implemented. In addition, the simplified version of the FINDRISC was developed with the data of this study, and not external validation was conducted. This could explain why diagnostic accuracy of this version of the FINDRISC was better than other scores. Therefore, further validation and evaluation is required.

9.3 Study relevance and implications

9.3.1 Blood-free risk scores and undiagnosed T2DM

The implementation of the FINDRISC in our population might be useful to detect cases with undiagnosed T2DM. The advantage of the FINDRISC lies on its self-report nature (six items with yes/no responses) and the presence of two anthropometrical measurements (body mass index and waist circumference). Our simplified version of the FINDRISC contains only three self-reported items and waist circumference, an anthropometric marker that is easy to obtain, making this score implementable in clinical practice. The simplified version of the FINDRISC included waist circumference instead of body mass index as in the Peruvian Risk Score, as the first one provides a better indicator of accumulation of visceral fat and glucose metabolism deregulation [59]. As one of the main barriers to the uptake of risk scores by health practitioners includes the lack of practicality of using the scores and evaluate their components [158], a short version of the FINDRISC might facilitate its implementation despite of the use of waist circumference instead of body mass index. A recent systematic review reported that financial constraints were one of the main barriers to T2DM screening in health system [159], issue that can be easily overcome by using a risk score. Moreover, the FINDRISC and the simplified FINDRISC can be easily self-administered.

The use of risk scores is only partly supported by different reports [160, 161] as there is no evidence of cardiovascular benefit. These recommendations are, however, based on the results of two trials [162, 163]. Although both trials had more than 10 years of follow-up, this can be a short time as the impact of a lifestyle intervention (Da Qing Diabetes Prevention Study) on all-cause and cardiovascular mortality of individuals with impaired glucose tolerance was detectable after 23 years of follow-up [164]. Thus, the benefit of T2DM screening needs to be further evaluated.

On the other hand, the potential effect of lifestyle interventions has only been demonstrated on those with impaired glucose tolerance and not among those with impaired fasting glucose alone [165, 166]. However, individuals with undiagnosed T2DM require lifestyle interventions but also medication to avoid or delay complications.

In 2016, the Peruvian Ministry of Health published the Guide of Clinical Practice for Diagnosis, Treatment and Control of Type 2 Diabetes Mellitus in Primary Care. In that guideline, there is no recommendation about the use of risk scores for T2DM screening, but, it recommends using fasting plasma glucose among adults between 40 and 70 years with overweight or obesity [167]. The FINDRISC, and also the simplified FINDRISC, appears then as good alternatives to screen individuals, especially in areas (semiurban and rural settings) where laboratory (fasting glucose or other blood markers) or human resources are not always available. It is still pending, nonetheless, to estimate the cost of a two-step approach for detecting cases of undiagnosed T2DM in resource-constrained settings, although some evidence in favour of multi-step approach exists [27].

9.3.2 Neuropathy assessment tools and undiagnosed T2DM

Among all the neuropathy assessments tool assessed in this study, the biothesiometer and the EZSCAN are the most likely to have some useful role in the detection of undiagnosed T2DM cases. However, based on sensitivity, the EZSCAN would perform better than the biothesiometer.

The biothesiometer, a device used to evaluate the vibration sensation, is very simple to use applying from 0 to 50 mV to a probe to increase vibration intensity especially in foot. As the test can be affected by individual's age, readings over 25 are considered as diagnostic of neuropathy [115, 116]. Although the pulp of hallux (toe) has been described as the best area to conduct the evaluation [116], our study reported that the third (or the fifth) metatarsal head has the best diagnostic accuracy for undiagnosed T2DM. On the other hand, the EZSCAN is a device based on the application of direct current through nickel electrodes on palms of the hands and soles of the feet due to the fact that sweat glands are very numerous in these areas [168]. Thus, the process of evaluation with EZSCAN is fast and, hence, only requires a short time with bare feet. Its described reproducibility makes this device an acceptable option as a screening tool for T2DM [100].

The impact of the implementation of these two neuropathy assessment tools to evaluate general population for looking cases of T2DM can be better appreciated using an example. In the case of the EZSCAN, using the cut-off of 26 as suggested by our analysis, from 1000 participants assessed, a total of 479 would be classified as having undiagnosed T2DM, with the subsequent detection of 65 cases and missing 45 (i.e. assuming a prevalence of T2DM of 11%). Thus, almost half of the participants (48%) assessed by EZSCAN will require a second test (i.e. fasting glucose, HbA1c or OGTT) to confirm EZSCAN findings. On the other hand, if we used a cut-off of 24 (to improve sensitivity), from 1000 screened subjects, 87 would be detected and only 23 would be missed. However, 692 (about 70%) individuals will require a second test to confirm T2DM. Thus, the change in the EZSCAN cut-off would impose an increment of 45% in the number of subjects to be tested after screening with the subsequent increase of costs and resources, but detecting 33% more T2DM cases. In the case of the biothesiometer, and using the vibration perception threshold of the third metatarsal head, from 1000 participants assessed, a total of 106 individuals would be classified as having undiagnosed T2DM, with the subsequent detection of only 34 cases and missing 76 (i.e. assuming a prevalence of T2DM of 11%). Thus, about 17% of the total participants assessed by the biothesiometer will require a second test.

9.3.3 Combination of risk scores and neuropathy assessment tools

One of the forms to improve diagnostic accuracy of different tests is the combination of them in a two-step approach [27, 118]; however, a balance between sensitivity and specificity is required. According to study results, the combination of FINDRISC and biothesiometer (vibration perception threshold in the third metatarsal head) can improve the diagnostic accuracy of risk scores and neuropathy assessment tools alone. The other combinations would require a confirmatory test in almost two thirds of the population instead of <50% as the proposed combination.

Again, an example can clarify the benefits of this approach (See Table IX-1 for details). If 1000 individuals were evaluated using a combination of FINDRISC and biothesiometer, 87 cases would be detected and 23 would be missing, assuming a T2DM prevalence of 11%. In addition, only 453 individuals would be classified as being at risk of T2DM (only 45% of the 1000 participants originally assessed). Thus, a confirmatory test (i.e. fasting glucose or glycated haemoglobin) would be carried out in <50% of the participants. If for example, a combination of FINDRISC and EZSCAN is used to detect cases of T2DM, a total of 625 individuals would need a confirmatory test (Table IX-1), an increment of 172 individuals with their respective costs and logistics, to detect only four more cases.

Regardless of whether biothesiometer or the EZSCAN is used, new studies are required to evaluate the cost for detecting one more case of T2DM in Peruvian and other resource-constrained settings. Despite the fact that a recent systematic review has reported that using a multi-step approach reduces the number needed to have the final diagnostic test for a definite diagnosis [27], and for instance, should reduce individual and health system expenses, the inclusion of the cost of neuropathy assessment tools needs to be included in estimations. Overall, biothesiometer is cheaper than the EZSCAN (USD 1500 vs. USD 18000, respectively), but the lifetime of these devices needs to be also considered.

Finally, it can be argued that positive and negative likelihood ratios found in this study are close to those related to minimal change in the likelihood of disease, as values >10 for LR+ and <0.1 for LR- are expected in this kind of studies [169]. However, the evaluation of such a kind of diagnostic test was not part of this study, instead of trying to find a real and implementable algorithm to detect cases of undiagnosed T2DM.

Whether a T2DM screening program is implemented, it is relevant to discuss positive and negative aspects of that decision. Among the positive aspects, a screening program as that proposed in the present study will reduce costs and can be massively implemented. Using scores and neuropathy assessment devices will reduce staff training (i.e. the EZSCAN is almost an automatic process) as well as the need of costs related to confirmation tests [170]. In addition, as pointed out by *Khunti et al* [27], responses rates will improve response rate to T2DM screening. Nevertheless, negative aspects need to be also highlighted. It has been described anxiety and mental health issues as there could be a delay in obtained final results [171, 172]. Time between result of the screening and confirmatory test should be reduced to the minimum to guarantee patient health. Although a trial has reported that early provision of test results did not have impact on patient reassurance [173], delays have been also related to abandon and lost to follow-up as lack of disease awareness is common especially in resource-constrained (i.e. rural or semiurban) settings.

9.4 Further steps in research

Potential areas for subsequent research derived from this study include:

- The evaluation of costs incurred when implementing a combination of risk scores and neuropathy assessment tools for detecting cases of T2DM.

- Determination of the lapse required for re-assessing participants, using our approach compared to risk scores alone or other combinations. Many studies are based on only one screening assessment and the potential impact on predicting T2DM.
- Implementation of the FINDRISC, neuropathy assessment tools, and combination of them for T2DM screening in primary healthcare facilities, and the potential impact on T2DM awareness, treatment, and control, as well as the incidence of micro- and macro-vascular complications, including cardiovascular death.
- The implementation of appropriate intervention strategies to reduce the burden of undiagnosed T2DM in resource-constrained settings, but also, to reduce complications, and improve treatment adherence.

9.5 Overall conclusions

The results of this thesis provide relevant information about the potential benefit of using risk score models, neuropathy assessment tools, and their combination to detect cases of undiagnosed T2DM. The combination of the FINDRISC score with the vibration perception threshold, obtained from the third metatarsal head, using a biothesiometer, can improve sensitivity of the FINDRISC and biothesiometer alone, without affecting specificity or the area under the ROC curve.

This thesis also confirmed the high burden of T2DM in Peru with 40% of participants unaware of their diagnosis. Our proposed approach could help tackle the burden of T2DM in semiurban and resource-constrained settings.

Table IX-1: Assessment of combinations of risk scores and neuropathy assessment tools

Combination	Sensitivity	Specificity	At high risk of T2DM	T2DM cases detected	Subjects without T2DM
EZSCAN with…					
FINDRISC	83.1%	40.2%	625 (62.5%)	91 (9.1%)	356 (35.6%)
LA-FINDRISC	85.9%	36.6%	658 (65.8%)	94 (9.4%)	326 (32.6%)
Simplified FINDRISC	93.0%	28.3%	740 (74.0%)	102 (10.2%)	252 (25.2%)
Biothesiometer with…					
FINDRISC	78.9%	58.9%	453 (45.3%)	87 (8.7%)	524 (52.4%)
LA-FINDRISC	78.9%	52.5%	510 (51.0%)	87 (8.7%)	467 (46.7%)
Simplified FINDRISC	90.1%	39.3%	639 (63.9%)	99 (9.9%)	350 (35.0%)

All the estimates were calculated assuming that 1000 individuals were screened and a prevalence of 11% of T2DM.

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LIST OF APPENDICES

APPENDIX A

Published paper:

Development and validation of a simple risk score for undiagnosed type 2 diabetes in a resource-constrained setting

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Research Article

Development and Validation of a Simple Risk Score for Undiagnosed Type 2 Diabetes in a Resource-Constrained Setting

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Objective. To develop and validate a risk score for detecting cases of undiagnosed diabetes in a resource-constrained country. *Methods*. Two population-based studies in Peruvian population aged ≥35 years were used in the analysis: the ENINBSC survey (n = 2,472) and the CRONICAS Cohort Study (n = 2,945). Fasting plasma glucose ≥7.0 mmol/L was used to diagnose diabetes in both studies. Coefficients for risk score were derived from the ENINBSC data and then the performance was validated using both baseline and follow-up data of the CRONICAS Cohort Study. *Results*. The prevalence of undiagnosed diabetes was 2.0% in the ENINBSC survey and 2.9% in the CRONICAS Cohort Study. Predictors of undiagnosed diabetes were age, diabetes in first-degree relatives, and waist circumference. Score values ranged from 0 to 4, with an optimal cutoff ≥2 and had a moderate performance when applied in the CRONICAS baseline data (AUC = 0.68; 95% CI: 0.62–0.73; sensitivity 70%; specificity 59%). When predicting incident cases, the AUC was 0.66 (95% CI: 0.61–0.71), with a sensitivity of 69% and specificity of 59%. *Conclusions*. A simple nonblood based risk score based on age, diabetes in first-degree relatives, and waist circumference can be used as a simple screening tool for undiagnosed and incident cases of diabetes in Peru.

1. Introduction

As of 2014, the worldwide prevalence of type 2 diabetes mellitus (T2DM) was estimated to be 9% among adults aged ≥18 years with great impact on mortality, particularly in low- and middle-income countries (LMIC) [1, 2]. Moreover, globally, approximately 25% to 75% of diabetes cases remain undiagnosed [3, 4], until further complications, especially at the macro- and micro-vascular level, manifest clinically. In Latin America, the proportion of undiagnosed diabetes at the population level ranged from 33% to 50% [5].

An important strategy to prevent or delay T2DM complications is the early identification of those with undiagnosed diabetes; yet, universal screening for diabetes at the population level is not practical in resource-limited settings. The American Diabetes Association recommends the use of glucose test as T2DM screening in people with overweight and obesity as well as in those with other risk factors [6]. As a result, risk assessment scores have been developed to address this problem in a simple and inexpensive way. Most of the available algorithms for diabetes screening have been developed in Caucasian [7–9] and Asian populations

[10–13] and very few in other ethnic groups [14, 15]. To date, one diabetes risk score has been developed and validated in Latin America so far which was derived from one urban area in Brazil [16], thus bearing limited generalizability to the wider region. Furthermore, it is well established that before adopting existing risk scores as screening tools in different populations and ethnic groups, their performance needs to be evaluated, calibrated, or validated in local settings [17].

As the American Diabetes Association, the Peruvian Ministry of Health recommends diabetes screening in general population with fasting glucose in adults aged 40 to 70 years with risk factors. However, fasting glucose is not always available in primary care settings, especially in semiurban and rural areas. As a result, a major challenge to be overcome in many countries is the implementation of a simple, fast, and laboratory-free based screening method.

Consequently, we aimed to develop a simple laboratory-free risk score to identify people with undiagnosed diabetes and incident diabetes in Peru, a Latin American country that spans coastal, Andean, and rainforest settings. In order to do so, this work benefited from two large-scale population-based surveys: the first one, representative at the national level, was used to develop the score, and the second one, a cohort study, was utilized for external validation.

2. Methods

2.1. Study Design and Participants. Two different population-based studies were used in this analysis. The *National Survey of Nutritional and Biochemical Indicators for Noncommunicable Diseases* (ENINBSC in Spanish), conducted by the Peruvian National Institute of Health [18], was used to develop our predictive model. This was complemented with the CRONICAS Cohort Study [19], whose baseline and longitudinal information was used to validate the risk score.

The ENINBSC is a national population-based survey carried out in Peru between August 2004 and April 2005, designed to estimate the prevalence of hypertension, type 2 diabetes mellitus, and other risk factors for noncommunicable diseases at the national and regional level [18]. Potential participants were those aged ≥20 years, habitual residents in the study area, and able to provide consent for their participation in the study. Pregnant women and those currently breastfeeding were excluded from the study. As per design, the ENINBSC sample was stratified according to Peru's five major regions of the country: Lima, rest of the Coast, urban Highlands, rural Highlands, and jungle. In each stratum, cluster of blocks were chosen using single random sampling techniques. Within each cluster, a random sample of households and participants were selected.

The CRONICAS Cohort Study is an ongoing cardiopulmonary project aimed to estimate the prevalence and incidence of hypertension, diabetes mellitus, and obesity in four different settings in Peru that differ in terms of their urbanicity and altitude: Pampas de San Juan de Miraflores, in the highly urbanized Lima, Puno in the altitude (3,825 meters above the sea level) contributing with rural and urban areas, and Tumbes, a semiurban area in the northern coast of Peru [19]. The study started in September 2010 and a follow-up visit was completed in March 2014. A sex- and age-stratified sample was selected at random for each of the settings and all participants aged ≥35 years, full time residents in the study area, and able to consent, were enrolled. Follow-up data used for this analysis was collected, on average, at 30 months after baseline.

2.2. Study Procedures. The procedures of the ENINBSC have been described previously [18]. Briefly, after consent, two different visits were scheduled. The first one lasted on average 40 minutes and was carried out to apply a face-to-face questionnaire regarding data about household characteristics, demographics, lifestyles behaviors, risk factors, and blood pressure measurements. The second visit lasted 30 minutes on average and was planned to have an appropriate period of fasting for blood sampling for glucose, total cholesterol, HDL-cholesterol, and the remaining anthropometric measures (height, weight, and waist circumference) using standard procedures.

Similarly, the procedures of the CRONICAS study has been published elsewhere [19]. In brief, participants responded to a face-to-face questionnaire applied by trained community health workers. Data collected comprised risk factors for cardiovascular disease based on a modified version of the WHO STEP approach questionnaire for surveillance of noncommunicable disease [20]. A period of 8 to 12 hours of fasting was required for blood sampling to collect fasting glucose, total cholesterol, and HDL-cholesterol. Height, weight, and waist circumference were also assessed, and blood pressure was measured in triplicate after five minutes of resting using an automatic monitor (OMRON HEM-780) previously validated in adult's population [21].

2.3. Variable Definitions. In both studies, diabetes was defined as any of the following conditions: fasting glucose ≥7.0 mmol/L (≥126 mg/dL) and/or self-report of physician diagnosis. Fasting glucose was assessed by an enzymatic colorimetric method (glucose oxidase GOD-PAP) in both studies. After excluding individuals without known diabetes, undiagnosed diabetes was also estimated to develop and validate the risk score [22].

Variables included in the analyses were built to guarantee similarities between both studies: sex; age (<55 and ≥55 years); education (in years); self-reported smoking (current versus never/former smoker); alcohol use (user versus never user); self-reported diabetes in first-degree relatives (participant's parents and/or siblings); and levels of physical activity (low versus moderate/high levels, based on the transport-related domain of the IPAQ). Anthropometric measurements included in the analysis were body mass index ((BMI), <25, 25–29.9, and ≥30 Kg/m^2), waist circumference (<90, 90–99.9, and ≥100 cm), waist-to-height ratio (<0.50, 0.50–0.59, 0.60–0.69, and ≥0.70) [23], and hypertension (measured or previously diagnosed) [24].

2.4. Statistical Analysis. A total of 4,206 participants were enrolled in the ENINBSC, but only 2,472 were included in this analysis. Reasons for exclusion were 1,524 because of age

<35 years to make both databases comparable, 129 because of no data about fasting plasma glucose levels being available, and 81 because of known diagnosis of diabetes. In the CRONICAS study, 3,601 participants were enrolled at baseline but only 2,948 records were analyzed as 465 had no data about glucose levels, and 188 were excluded because of previous diagnosis of diabetes. In addition, only data from 2,577 participants was used in the longitudinal assessment of the risk score (comparison of baseline characteristics among those included and excluded from longitudinal analysis is shown in Online Supplement: E-Table 1; see Supplementary Material available online at http://dx.doi.org/10.1155/2016/8790235).

Initially, population characteristics of both studies were tabulated using proportions in the case of categorical variables and means and standard deviation (SD) with numerical variables. Then, the prevalence and 95% confidence intervals (95% CI) of total diabetes and undiagnosed diabetes were estimated in each study. After that, all cases of known diabetes were excluded from subsequent analyses.

The risk score was derived from data of the ENINBSC survey taking into account the multistage sampling strategy of the study. Each potential risk factor (i.e., sex, age, family history of diabetes, etc.) was assessed in bivariate models using logistic regression and undiagnosed diabetes as the dependent variable. Then, risk factors with a p value <0.10 in the bivariate analysis were included in a multiple logistic regression model using stepwise backward elimination with a significance level of 5%. The Hosmer-Lemeshow goodness-of-fit test was used to assess how well the predicted prevalence matched the observed prevalence of undiagnosed diabetes (i.e., p values over 0.20 indicate that model fits well) [25]. As we sought for an easily applicable and implementable algorithm, the risk factors in the final model were each assigned a weighted score by rounding up all regression coefficients in the final model to the nearest integer as in a previous report [26].

For the evaluation of the risk score, the area under the receiver operating characteristic (ROC) curve, sensitivity, specificity, and positive and negative predictive values (PPV and NPV) were calculated. The optimal cut-point was determined using the Youden index, a single statistic that captures the performance of a diagnostic test (i.e., sensitivity + specificity − 1) [27]. As one of the main aims of a nonlaboratory risk score is to identify people who warrant having a blood test (i.e., fasting glucose, glycated haemoglobin, etc.), the cut-point with the highest sensitivity was also estimated and described.

We assessed the performance of our score using bootstrap techniques as well as carrying out an external validation using the CRONICAS Cohort Study. Bootstrapping was utilized to estimate confidence intervals for the AUC in our study population. A total of 1,000 random samples with replacement were taken from the development database. The resulting 1,000 prediction models were then assessed to estimate the bootstrap AUC using the bias-corrected version of the confidence intervals [28]. In addition, using baseline data from the CRONICAS Cohort Study, validation measures (AUC, sensitivity, specificity, predictive values, and likelihood ratios) were estimated.

To evaluate the performance of our algorithm, the Peruvian risk score was compared to previously published models for undiagnosed diabetes including the Brazilian risk score [16], the Qingdao score [10], the Indian risk score [11], the Kuwaiti risk score [29], the patient self-assessment score [26], and the Rotterdam risk score [7] using the c-statistic. Finally, using the follow-up data of the CRONICAS Cohort Study, the risk score was also evaluated to detect incident cases of T2DM by excluding those with diabetes diagnosis at baseline. Analyses were performed using STATA 13.0 (StataCorp, College Station, TX, USA).

2.5. Ethical Issues. The protocol and informed consent forms of the ENINBSC study were reviewed and approved by the *Instituto Nacional de Salud* and the *Centro Nacional de Alimentación y Nutrición*, both part of the Ministry of Health in Lima, Peru. In the case of the CRONICAS Cohort Study, protocol and consent forms were reviewed and approved by the institutional review boards of the Universidad Peruana Cayetano Heredia and the NGO Asociación Benéfica PRISMA in Lima, Peru, and the Johns Hopkins University in Baltimore, USA.

3. Results

The characteristics of participants in both studies are detailed in Table 1. Overall, participants from the CRONICAS study were 5 years older, reported consuming lower levels of alcohol, and were less physically active than those from the ENINBSC survey.

3.1. Prevalence of Diabetes and Undiagnosed Diabetes. The overall prevalence of diabetes was 5.1% (129/2538; 95% CI: 4.2%–5.9%) in the ENINBSC survey and 8.7% (272/3135; 95% CI: 7.7%–9.7%) in the CRONICAS Cohort Study's baseline. After excluding those with known diabetes, undiagnosed diabetes was present in 2.0% (48/2457; 95% CI: 1.4%–2.5%) in the ENINBSC survey and in 2.9% (85/2948; 95% CI: 2.3%–3.5%) in the CRONICAS Cohort Study.

3.2. Development of the Risk Score. After stepwise backward logistic regression, age, diabetes in first-degree relatives, and waist circumference were independently associated with undiagnosed diabetes (Table 2). The Hosmer-Lemeshow test showed that the final model fitted relatively well ($p = 0.21$). The Peruvian diabetes risk score was constructed based on the coefficients of that final regression model. The score gave an AUC of 0.73 (95% CI: 0.65–0.78), and the optimal cut-point for undiagnosed diabetes using the Youden index was ≥2 (Figure 1). With this cut-point, about 34.8% of participants were categorized as at high risk of diabetes: sensitivity 69.6%, specificity 65.8%, and PPV and NPV of 3.9% and 99.1%, respectively. With a cut-point ≥1, 69.8% of participants would be at high risk of diabetes with improved sensitivity (93.5%) but lower specificity (30.6%). Table 3 shows the performance of the risk score for detecting undiagnosed diabetes at different cut-points.

Table 1: Sociodemographic characteristics of participants without history of type 2 diabetes in the two involved studies.

	ENINBSC study ($n = 2{,}472$)	CRONICAS study ($n = 2{,}945$)
Demographic variables		
Sex (% females)	1,209 (48.9%)	1,500 (50.9%)
Age (mean (SD))	50.5 (12.1)	55.3 (12.7)
Education in years (mean (SD))	7.8 (4.9)	8.0 (4.9)
Behavioural variables		
Current smoking (%)	391 (15.9%)	369 (11.5%)
Alcohol use (%)	2,323 (94.1%)	1,600 (54.3%)
Family history of diabetes (%)	268 (11.2%)	351 (11.9%)
Physical activity (% low level)	606 (24.5%)	938 (31.9%)
Anthropometric measures		
Body mass index (mean (SD))	25.7 (4.5)	27.6 (4.6)
Waist circumference (mean (SD))	91.0 (11.4)	91.5 (11.0)
Waist-to-height ratio (mean (SD))	0.58 (0.08)	0.59 (0.07)
Systolic blood pressure (mean (SD))	114.5 (18.5)	117.2 (18.9)
Diastolic blood pressure (mean (SD))	71.1 (11.9)	73.4 (11.1)
Hypertension (%)	579 (23.8%)	705 (24.0%)
Total cholesterol (mean (SD))	174.2 (36.9)	199.7 (39.6)
HDL-cholesterol (mean (SD))	43.5 (5.3)	41.7 (11.5)

SD: standard deviation and HDL: high-density lipoprotein.
Results may not add due to missing values.

Table 2: Risk factors and beta coefficients for undiagnosed diabetes: final regression model using CENAN database ($n = 2{,}367$).

	Bivariate model		Final model*		Score
	Coefficient (SE)	OR (95% CI)	Coefficient (SE)	OR (95% CI)	
Sex					
Male (versus female)	−0.39 (0.30)	0.68 (0.38–1.21)			
Age					
≥55 (versus <55 years)	0.72 (0.29)	2.05 (1.16–3.64)	0.61 (0.18)	1.85 (1.30–2.63)	1 (versus 0)
Current smoking					
Current (versus never/former smoker)	−1.06 (0.60)	0.34 (0.11–1.12)			
Alcohol user					
User (versus never user)	0.38 (0.74)	1.46 (0.34–6.27)			
Diabetes in relatives					
Yes (versus no)	1.06 (0.34)	2.90 (1.48–5.66)	0.85 (0.42)	2.34 (1.04–5.31)	1 (versus 0)
Physical activity					
Low (versus moderate/high levels)	0.80 (0.30)	2.24 (1.25–4.01)			
Body mass index					
Overweight (versus normal)	0.07 (0.35)	1.07 (0.54–2.13)			
Obese (versus normal)	0.80 (0.36)	2.23 (1.11–4.49)			
Waist circumference					
90.0 to <99.9 cm (versus <90 cm)	0.66 (0.38)	1.93 (0.91–4.10)	0.74 (0.33)	2.09 (1.09–4.02)	1 (versus 0)
100+ cm (versus <90 cm)	1.41 (0.37)	4.10 (1.99–8.44)	1.40 (0.23)	4.07 (2.60–6.40)	2 (versus 0)
Waist-to-height ratio					
0.50–0.59 (versus <0.50)	0.34 (0.63)	1.41 (0.41–4.86)			
0.60–0.69 (versus <0.50)	1.09 (0.62)	2.97 (0.88–10.0)			
0.70+ (versus <0.50)	1.58 (0.68)	4.84 (1.27–18.5)			
Hypertension					
Yes (versus no)	0.52 (0.31)	1.68 (0.91–3.09)			

*The model was created using backward elimination from the initial full model until we reached a final model with statistically significant covariates.

Table 3: Performance of different cut-points for detecting undiagnosed type 2 diabetes in the development database.

Total score	At high risk*	Sensitivity	Specificity	PPV	NPV	Correctly classified	LR+	LR−
≥1	69.8%	93.5%	30.6%	2.6%	99.6%	31.8%	1.34	0.21
≥2	34.9%	69.6%	65.8%	3.9%	99.1%	65.9%	2.04	0.46
≥3	11.0%	30.4%	89.4%	5.4%	98.5%	88.3%	2.87	0.78
≥4	1.3%	2.2%	98.7%	3.2%	98.1%	96.8%	1.68	0.99

PPV: positive predictive value; NPV: negative predictive value; LR+: positive likelihood ratio; LR−: negative likelihood ratio.
*Those at high risk are the proportion of participants over the total score.

Table 4: Performance of different diabetes risk scores compared to Peruvian diabetes risk score using the CRONICAS study (validation sample).

Method (proposed cutoff)	# of variables	AUC	Sensitivity	Specificity	PPV	NPV	LR+	LR−
Brazilian risk score (≥18)	3	0.65	66.7%	61.9%	4.9%	98.4%	1.75	0.54
Qingdao risk score (≥17 and ≥14)*	4	0.58	83.3%	33.3%	3.6%	98.5%	1.25	0.50
Indian risk score (≥21)	5	0.54	94.0%	15.5%	3.1%	98.9%	1.11	0.39
Kuwaiti risk score (≥32)	4	0.62	45.2%	78.4%	5.8%	98.0%	2.09	0.70
Patient self-assessment score (≥5)	6	0.64	61.4%	66.8%	5.1%	98.3%	1.85	0.58
Rotterdam risk score (≥36)	6	0.55	94.0%	16.8%	3.2%	99.0%	1.13	0.35
Peruvian risk score (≥2)	3	0.68	70.2%	58.9%	4.8%	98.5%	1.71	0.51

AUC: area under the ROC curve; PPV: positive predictive value; NPV: negative predictive value; LR+: positive likelihood ratio; LR−: negative likelihood ratio.
*Different cutoffs for males (≥17) and females (≥14).

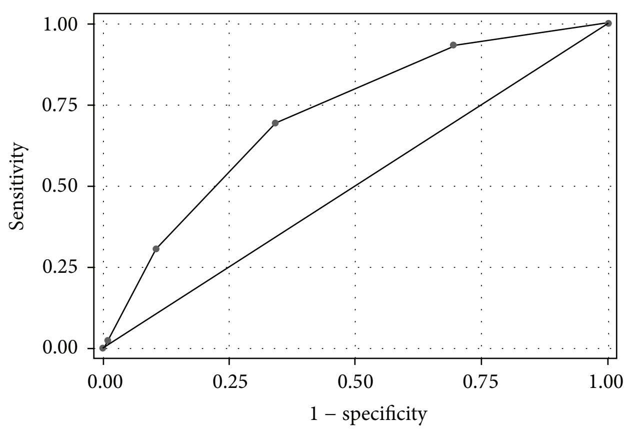

Figure 1: Receiver operating characteristic (ROC) curve of the risk score in predicting undiagnosed type 2 diabetes in the development database. The area under the ROC curve was 0.73 (95% CI: 0.65–0.78) for the risk score.

3.3. Cross-Sectional Validation of the Risk Score. When bootstrap was used, the performance of our risk score was similar to the obtained in the development model (AUC = 0.72; 95% CI: 0.65–0.78). In addition, when the risk score was evaluated by applying the score to the CRONICAS Cohort Study's population, the AUC for undiagnosed diabetes was 0.68 (95% CI: 0.62–0.73). At the suggested cut-point of ≥2, 42% would be categorized as undiagnosed diabetes with sensitivity, specificity, PPV, and NPV of 70.2%, 58.9%, 4.8%, and 98.5%, respectively (Table 4). On the other hand, with a cut-point ≥1, 80% would be categorized as undiagnosed diabetes with sensitivity, specificity, PPV, and NPV of 94.0%, 20.0%, 3.3%, and 99.1%, respectively.

When previous published algorithms for undiagnosed diabetes were applied to the CRONICAS Cohort Study, the performance of the Rotterdam score ($p < 0.001$), Indian score ($p < 0.001$), and Qingdao score ($p < 0.01$) was poorer than our score; however, our algorithm performed similar to the other assessed models, such as the Brazilian risk score ($p = 0.93$), the Kuwaiti score ($p = 0.26$), and the patient self-assessment score ($p = 0.74$), but having only three variables.

3.4. Longitudinal Assessment of the Risk Score. The performance of this risk score was also assessed to predict incident cases of diabetes using the longitudinal data from the CRONICAS Cohort Study. One hundred twenty-one new cases of diabetes were found accounting for 6,207 person-years at risk, with an overall incidence of 1.95 (95% CI: 1.63–2.33) cases per 100 person-years of risk. The AUC of the score was 0.66 (95% CI: 0.61–0.71). With a cut-point ≥2, 42.5% of participants were categorized as at high risk of developing diabetes: sensitivity, specificity, PPV, and NPV were 69.4%, 58.9%, 7.8%, and 97.4%, whereas, for a cut-point ≥1, the respective values were 79.9%, 91.9%, 20.7%, 5.5%, and 98.1%.

4. Discussion

4.1. Main Findings. Using a national population-based survey, a simple nonblood based risk score based on age, history of diabetes in first-degree relatives, and waist circumference was built and shown to perform moderately in detecting undiagnosed diabetes when externally validated. Moreover, the performance of the score was almost similar for detecting incident cases of diabetes in the Peruvian population.

4.2. Comparison with Other Risk Scores. A relatively recent systematic literature search found 23 different blood-free prevalent diabetes risk scores: ten from Europe, nine for Asian populations, two from the United States, and two from Middle East [30]. In addition, and not included in the aforementioned review, only one risk score was developed in Latin America using Brazilian urban population [16]. The same systematic review reported that AUC for these predictive models was greater in the development studies (range: 0.65 to 0.88) than in the validation studies (range: 0.63 to 0.80) [30], similar to our findings. Another systematic review found that several noninvasive algorithms were created using variables such as age, gender, waist circumference and/or BMI, and family history of diabetes in the final model [31]. As impracticality due to use of the algorithms was a common barrier to the uptake of risk scores by healthcare staff and individuals [32], our model, created with three of these more common variables, reached a moderate-to-high sensitivity depending on the used cut-point. Moreover, two of these variables are easily evaluable during medical appointment or through individual's self-assessment, and only a measuring tape and no calculations are required to be implemented in clinical practice or at the population level.

From a cross-sectional point of view, with a cut-point ≥2, from 1000 participants assessed by the Peruvian diabetes risk score, a total of 420 would be classified as undiagnosed diabetes with the detection of 20 cases and only 6 will be missing. On the other hand, with a cut-point ≥1, from 1000 screened individuals, a total of 804 would be categorized as having undiagnosed diabetes with the detection of 27 cases and only 7 will be missing. Thus, the reduction of the cut-point of the risk score would increase sensitivity but reducing the specificity and imposing the need of performing a confirmatory test (i.e., fasting glucose) to almost the double of individuals, with the benefit of having only 7 more people diagnosed.

Longitudinally, the same risk score would detect an important number of participants at risk of developing diabetes: 43% of screened individuals would be classified at high risk of diabetes, and of them, 8% would develop diabetes in the next 2.5 years. According to a previous study [33], 17 reports described a noninvasive model to predict the development of diabetes and included a median of six risk predictors, ranging from 2 to 11 [34]. Although our score did not perform as good as other well-known longitudinal models in the literature such as the FINDRISC or the ARIC scores [35, 36], it only included three variables and was built using cross-sectional information. In addition, some variables used in the aforementioned studies are difficult to standardize within a country as Peru, that is, food portions, physical activity, or sedentarism, limiting therefore its use on a wider scale and in a simple pragmatic fashion.

Our algorithm performed better than the Rotterdam, the Indian, and the Qingdao risk scores in our population, which highlights the need of calibration and/or development of a specific score for different ethnic groups before its adoption. As there are ethnic differences in risk factors for diabetes and Peru is considered a multiethnic country, it is necessary to create specific scores or recalibrate existing algorithms before applying in specific contexts. In addition, with only three variables included, the performance of our predictive model was similar to the other assessed scores included in the analyses. Taken together, the score developed has the potential to augment, in a pragmatic manner, initial rapid screening for diabetes, especially at various nonspecialized primary healthcare services.

Our findings also demonstrate that approximately 35% of cases of T2DM (39% in the ENINBSC survey and 33% in the baseline of the CRONICAS Cohort Study) are not aware of their disease. Results are similar to those reported in previous studies in our context [37] and in similar settings in Latin America [38].

4.3. Public Health Relevance and Implications. As the developed risk score is simple, it does not require a blood test or laboratory services, and it might be easily implemented in clinical practice. Moreover, because our score asks for general information in the form of age and diabetes in first-degree relatives and is complemented by a simple anthropometric measure of waist, there is potential for the score to be self-administered.

According to our results, any patient aged 55 years and above and having at least one first-degree relative with T2DM has greater probability of having undiagnosed diabetes but also is at risk of developing diabetes in the future. In addition, a greater central obesity, that is, 100 cm or more, independent of the other terms of the score is alone a good predictor of diabetes as reported in previous studies [23]. Our algorithm included waist circumference instead of body mass index as other risk scores, providing a better indicator of accumulation of visceral fat and metabolic dysfunction in our context [39].

Recently, the Peruvian Ministry of Health has published the Guide of Clinical Practice for Diagnosis, Treatment and Control of Diabetes Mellitus in Primary Care [40] and only recommends screening in general population with plasma glucose among adults between 40 and 70 years with obesity or overweight as suggested by the American Diabetes Association [6]. As in other LMIC, plasma glucose is not always available in primary care, especially in semiurban and rural areas; therefore, a major challenge to be overcome in many countries is the implementation of a simple, fast, and laboratory-free based screening method. Moreover, within the Peruvian context, no risk score has been proposed as part of the aforementioned guide. Thus, our algorithm might fill a gap to facilitate further specialized assessment of high risk individuals for diabetes, an approach that may be of utility to various other countries facing similar challenges.

4.4. Strengths and Limitations. The strengths of this study include the use of a national population-based survey, including urban and rural areas across major geographical regions, to develop the Peruvian diabetes risk score, as well as its validation using bootstrap but also an independent longitudinal cohort study. Additionally, it is only based on three variables ensuring its simplicity to be used and implemented. However, the study has also some limitations. First, we have utilized fasting plasma glucose as the gold standard for diagnosing diabetes instead of an oral glucose tolerance test (OGTT). Although the OGTT is more sensitive and specific than the fasting plasma glucose, more cases would have been detected with the overload of glucose; it is rarely performed as part of the routine clinical practice. Second, the CRONICAS Cohort Study did not include information from the Amazon rainforest as did the ENINBSC survey. When a sensitivity analysis was performed excluding individuals from the jungle from ENINBSC data, results were similar to those presented in this manuscript (data not shown). In addition, the score was created using a national survey to be applicable to the entire Peruvian population. Third, some variables were not assessed in our logistic regression model such as dietary intake or history of gestational diabetes as such data was not available. As a result, some caution should be made when our algorithm is compared to other risk scores. Fourth, our model is based on the idea of risk stratification instead of individualisation [41]; for instance, variables were categorized instead of being preserved as numerical. Nevertheless, the performance of our score did not change when age and waist circumference were treated as numerical variables (data not shown). Moreover, our idea was to develop a simple and easily applicable score instead of a complex algorithm for predicting undiagnosed and incident diabetes. Finally, as other diabetes risk scores, the model warrants further scrutiny before it can be used in other populations.

5. Conclusions

The Peruvian diabetes risk score, built using age, self-reported diabetes in first-degree relatives, and waist circumference, proves to be a simple pragmatic screening tool for undiagnosed and incident cases of diabetes in Peru. This experience in generating such simple, easy-to-use approaches for the identification of T2DM can serve to inform other similar LMIC efforts who are on early stages of diabetes prevention. This tool, due to its simplicity, can facilitate various initiatives oriented to introduce and scale up early preventative and management strategies on a wider scale.

Competing Interests

The authors declare that there are no competing interests.

Authors' Contributions

Antonio Bernabe-Ortiz, Liam Smeeth, and J. Jaime Miranda conceived the idea of the manuscript. Antonio Bernabe-Ortiz drafted the first version of the manuscript and led the statistical analysis. Jose R. Sanchez-Abanto supervised the ENINBSC survey. J. Jaime Miranda, Liam Smeeth, Robert H. Gilman, and William Checkley conceived, designed, and supervised the overall CRONICAS Cohort Study. All authors participated in manuscript writing, provided important intellectual content, and gave their final approval of the version submitted for publication.

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APPENDIX B

Published paper:

EZSCAN for undiagnosed type 2 diabetes mellitus: A systematic review and meta-analysis

PLoS One 2017; 12(10): e0187297

RESEARCH ARTICLE

EZSCAN for undiagnosed type 2 diabetes mellitus: A systematic review and meta-analysis

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Abstract

Objectives

The EZSCAN is a non-invasive device that, by evaluating sweat gland function, may detect subjects with type 2 diabetes mellitus (T2DM). The aim of the study was to conduct a systematic review and meta-analysis including studies assessing the performance of the EZSCAN for detecting cases of undiagnosed T2DM.

Methodology/Principal findings

We searched for observational studies including diagnostic accuracy and performance results assessing EZSCAN for detecting cases of undiagnosed T2DM. OVID (Medline, Embase, Global Health), CINAHL and SCOPUS databases, plus secondary resources, were searched until March 29, 2017. The following keywords were utilized for the systematic searching: type 2 diabetes mellitus, hyperglycemia, EZSCAN, SUDOSCAN, and sudomotor function. Two investigators extracted the information for meta-analysis and assessed the quality of the data using the Revised Version of the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) checklist. Pooled estimates were obtained by fitting the logistic-normal random-effects model without covariates but random intercepts and using the Freeman-Tukey Arcsine Transformation to stabilize variances. Heterogeneity was also assessed using the I^2 measure. Four studies (n = 7,720) were included, three of them used oral glucose tolerance test as the gold standard. Using Hierarchical Summary Receiver Operating Characteristic model, summary sensitivity was 72.0% (95%CI: 60.0%– 83.0%), whereas specificity was 56.0% (95%CI: 38.0%– 74.0%). Studies were very heterogeneous (I^2 for sensitivity: 79.2% and for specificity: 99.1%) regarding the inclusion criteria and bias was present mainly due to participants selection.

Conclusions

The sensitivity of EZSCAN for detecting cases of undiagnosed T2DM seems to be acceptable, but evidence of high heterogeneity and participant selection bias was detected in most

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of the studies included. More studies are needed to evaluate the performance of the EZSCAN for undiagnosed T2DM screening, especially at the population level.

Introduction

Worldwide, the burden of type 2 diabetes mellitus (T2DM) is rising rapidly. Currently, approximately 9% of adults in the world are living with T2DM [1, 2]. Many of the consequences of T2DM affect mainly low- and middle-income countries (LMIC): 1.5 million deaths worldwide were directly attributable to T2DM in 2012, and more than 80% of these deaths occurred in LMIC [3, 4]. In addition, about USD$ 548 billion in healthcare expenditures were due to T2DM in 2013 [5], imposing a large economic burden on individuals and families as well as health systems, particularly in resource-constrained settings.

Oral glucose tolerance test (OGTT) is considered the gold standard for T2DM diagnosis according to guidelines [6]. However, conventionally, fasting glucose (FG) is used in most of healthcare facilities. OGTT and FG require 8 hours of fasting and, in addition, OGTT also needs the participant to drink a 75-gram glucose solution and wait two hours before a second blood sample is obtained. In 2009, the American Diabetes Association suggested that glycated hemoglobin (HbA1c) could be used as a diagnostic tool for T2DM [7]. HbA1c does not require fasting, but can be expensive and requires a certified laboratory process [8]. Despite the recommended cutoff of 6.5% (48 mmol/mol) for T2DM diagnosis [9], discrepancies between HbA1c and glycemia in different racial and ethnic groups have been described [10–13].

An important approach to prevent or delay diabetes complications is to identify those individuals with undiagnosed T2DM [14]. Although universal T2DM screening at the population level is not practical; there are alternative methods reported in the literature. As early damage of small nerves can be found since the onset of T2DM [15], some devices have emerged to assess small-fiber autonomic dysfunction [16]. Among these devices, the EZSCAN (Impeto Medical, Paris, France), a non-invasive device that performs electrochemical reactions of eccrine sweat glands, may help to detect participants with diabetes mellitus [17, 18]. The advantage of the EZSCAN is that its use does not require trained personnel, delivers result quickly, and does not require active participation of the participants (i.e. fasting). Some studies have evaluated the potential impact of this device in pre-diabetes, dysglycemia and T2DM screening [17, 19, 20], but there is limited information regarding its potential for detecting cases of undiagnosed T2DM. Consequently, we conducted a systematic review and meta-analysis of observational studies to assess the performance of the EZSCAN for undiagnosed T2DM. Our hypothesis was focused on sensitivity, expecting at least a performance of 75%.

Materials and methods

Study selection

We searched for observational studies including diagnostic accuracy results assessing EZSCAN for undiagnosed T2DM, conducted in different parts of the world, but reported in English. Studies were excluded if they were only abstracts or review articles, enrolled individuals aged <18 years or cases with type 1 diabetes mellitus, and defined type 2 diabetes mellitus (T2DM) by using blood markers other than OGTT or FG (i.e. HbA1c). The rationale for this decision was based on discrepancies between HbA1c and glycemia in different racial and ethnic groups and that HbA1c is not commonly used for undiagnosed T2DM.

Data sources and searches

A comprehensive literature search using the Ovid database (PubMed-Medline, Embase, Global Health, and Health Management Information Consortium) as well as CINAHL, and SCOPUS, until March 29, 2017, was conducted. The following keywords were utilized for the systematic searching: type 2 diabetes mellitus, hyperglycemia, EZSCAN, SUDOSCAN, and sudomotor function [16]. The term SUDOSCAN was also included in the search strategy as it uses the same principle (i.e. sudomotor function assessment) for detecting diabetic neuropathy [21, 22]. The search strategy of Ovid is available in S1 Table. The Impeto Medical website was also searched to find other published manuscripts [19].

Data extraction and quality assessment

Titles and abstracts of retrieved articles were reviewed independently by two investigators to select potentially relevant articles, and disagreements were discussed and solved by consensus. Using a standardized data extraction form, we collected information on lead author, publication year, country, study design, inclusion criteria, used gold standard, sample size, mean age, percentage of male participants, and different indicators of the performance of the EZSCAN to detect undiagnosed T2DM (outcome, area under the curve, cut-off, sensitivity, specificity, among others).

Quality assessment of individual studies was performed to identify potential sources of bias and to limit, if possible, the effect of these biases on the conclusions of the review. For this, the Revised Version of the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) checklist was used [23]. This tool included risk of bias assessment (i.e. participant selection, index test, reference standard, and flow and timing) as well as applicability.

Data synthesis and analysis

The primary outcome of interest was undiagnosed T2DM (i.e. newly-diagnosed T2DM) identified by OGTT or FG. Secondary outcomes included other glucose metabolism disorders such as impaired glucose tolerance, impaired fasting glucose and, dysglycemia.

Statistical analyses were performed using Stata version 13 for Windows (StataCorp, College Station, TX, US). Our systematic review followed the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA, See S1 Checklist), the Guidelines for Meta-Analyses and Systematic Reviews of Observational Studies (MOOSE) [24] as well as the Cochrane Handbook for Diagnostic Test Accuracy Reviews [25]. Initially, the studies included in the systematic review were described, including: publication year, country, study design, inclusion criteria, gold standard, sample size, mean age, and proportion of males. In addition, the performance of the EZSCAN in each study was tabulated, and the area under the receiver operating characteristic (ROC) curve (AUC), best cut-off, sensitivity, and specificity, and their respective 95% confidence intervals (95%CI) were reported, if available.

A meta-analysis of the performance of the EZSCAN was conducted using data from studies with undiagnosed T2DM as outcome. Information used in meta-analysis was taken as proposed by manuscripts according to the best EZSCAN threshold cut-off reported. The "*metaprop*" command in STATA was used to estimate sensitivity, specificity and positive (PPV) and negative (NPV) predictive values and their respective 95%CI [26]. The "*metaprop*" command obtains a pooled estimate as a weighted average, by fitting the logistic-normal random-effects model without covariates but random intercepts. The pooled estimate was then calculated using the Freeman-Tukey Arcsine Transformation to stabilize the variances as suggested in literature [27]. In addition, a graph containing the plot of the Hierarchical Summary Receiver Operating Characteristic (HS-ROC) model [28], a summary point of sensitivity and specificity

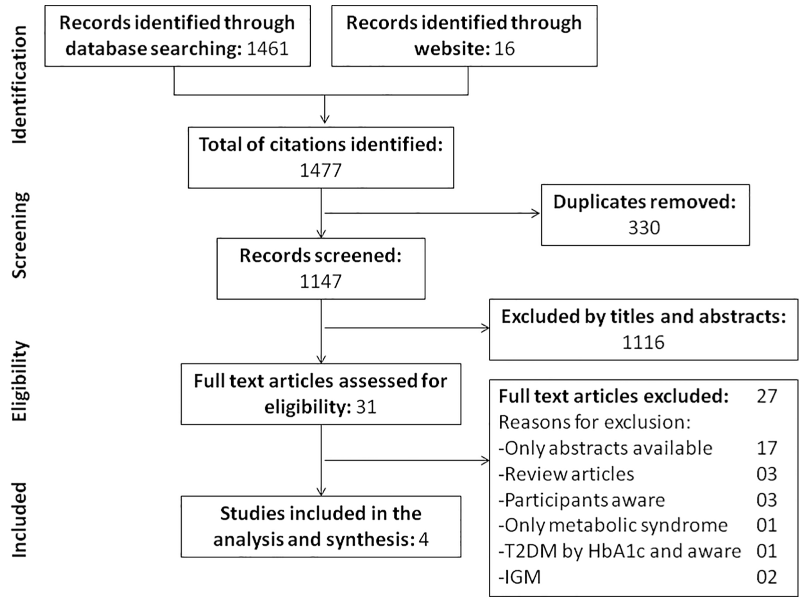

Fig 1. Flowchart of database searches and articles included in the systematic review. T2DM: Type 2 diabetes mellitus, HbA1c = glycated hemoglobin, IGM = impaired glucose metabolism.

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and the 95% confidence region for that point was obtained by using the "*metandiplot*" command [29]. Heterogeneity of estimates and 95%CI was determined using the I^2 measure [30].

Results

Study characteristics

A total of 1,461 citations were identified through our systematic search, with a further 16 citations identified using the Impeto Medical website. After excluding duplicates (n = 330), a total of 1,147 citations were independently screened, of which 31 were retrieved for detailed assessment (agreement between reviewers, 97.2%, kappa = 0.61, p<0.001). Of the 31 revised manuscripts, 27 did not fit our inclusion criteria (Fig 1); therefore, four studies were included in the systematic review.

The characteristics of the studies included in the systematic review are shown in Table 1. All the four studies were cross-sectional in nature. A total of 7,720 individuals were included

Table 1. Characteristics of the studies included in the systematic review.

Study, publication year	Country	Study design	Inclusion criteria	Gold standard	Size	Mean age	% male
Chen X, 2015 [32]	China	Cross-sectional	Subjects in routine health check visiting a Community Hospital, at risk of T2DM (age ≥ 45 years).	OGTT	270	58.6	32%
Ramachadran A, 2010 [33]	India	Cross-sectional	Individuals in specific clinics aged between 21–75 years.	OGTT	212	43.4	45%
Sanchez-Hernandez O, 2015 [34]	Mexico	Cross-sectional	Individuals recruited in a clinic in Mexico; ≥18 years, apparently healthy and attending a full check-up.	FG	1,414	44.7	50%
Yang Z, 2013 [31]	China	Cross-sectional	Individuals from two communities in Shanghai aged 40 + years.	OGTT	5,824	58.3	40%

FG = fasting glucose; OGTT = oral glucose tolerance test.

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from all the studies, but 5,824 subjects came from a single study [31]. This latter study enrolled individuals from the general population, whereas the remaining three studies recruited participants at clinics, mainly individuals going for healthy check-ups.

Risk of bias

Overall, participant selection bias was present in 3 out of 4 of the studies included in the meta-analysis [32–34]: individuals under healthy check-ups were enrolled in the original studies (S2 Table). In addition, flow and timing was unclear in the same three studies, and the gold standard (i.e. OGTT) was not used in one of the studies [34].

Meta-analysis: EZSCAN performance for undiagnosed T2DM

Undiagnosed T2DM was the outcome of interest in the four studies (Table 2). Other outcomes evaluated in these papers included impaired glucose tolerance [32, 33], impaired fasting glucose [34] and dysglycemia [31].

When undiagnosed T2DM was the outcome, only two studies reported results of AUC ranging from 53% to 73% [32, 34]. In addition, 2 studies used 50% as the suggested EZSCAN cut-off for undiagnosed T2DM screening [32, 33], whereas one used 34% [34], and the last one utilized 30% [31]. Sensitivity varied from 53% to 81%, whilst specificity ranged from 43% to 70%. Finally, positive predictive values (PPV) varied from 10% to 40%, whereas negative predictive values (NPV) ranged from 71% to 98%.

When using HS-ROC (Fig 2), summary sensitivity was 72.0% (95%CI: 60.0%– 83.0%), specificity was 56.0% (95%CI: 38.0%– 74.0%), PPV was 24% (95%CI: 12.0%– 37.0%), and NPV was 89% (95%CI: 82.0%– 97.0%). In addition, positive and negative likelihood ratios were 1.68 (95%CI: 1.35–2.10) and 0.48 (95%CI: 0.36–0.66), respectively, whereas the DOR was 3.49 (95% CI: 2.18–5.57). Heterogeneity for sensitivity was 79.2% (95%CI: 44.0%– 92.0%), whereas for specificity was 99.1% (95%CI: 98.5%– 99.6%).

Table 2. Performance of the EZScan in the studies included in the systematic review.

Study, publication year	Outcome	AUC	Cut-off	Sensitivity	Specificity
Chen X, 2015 [32]	IGT	78% (72%–83%)	37%	82% (72%–90%)	63% (55%–71%)
Chen X, 2015 [32]	T2DM	53% (43%–62%)	50%	53% (36%–69%)	59% (47%–70%)
Ramachadran A, 2010 [33]	IGT	—	50%	70% (not reported)	54% (not reported)
Ramachadran A, 2010 [33]	T2DM	—	50%	75% (not reported)	54% (not reported)
Sanchez-Hernandez O, 2015 [34]	IFG	65% (not reported)	27%	69% (not reported)	56% (not reported)
Sanchez-Hernandez O, 2015 [34]	T2DM	73% (not reported)	34%	73% (not reported)	70% (not reported)
Yang Z, 2013 [31]	IFG, IGT or T2DM	—	30%	73% (71%-75%)	46% (45%-48%)
Yang Z, 2013 [31]	T2DM	—	30%	81% (78%-83%)	43% (42%-44%)

IFG = Impaired fasting glucose; IGT = Impaired glucose tolerance; T2DM = type 2 diabetes mellitus; AUC = area under the curve.
Values in brackets are 95% confidence intervals (95%CI).

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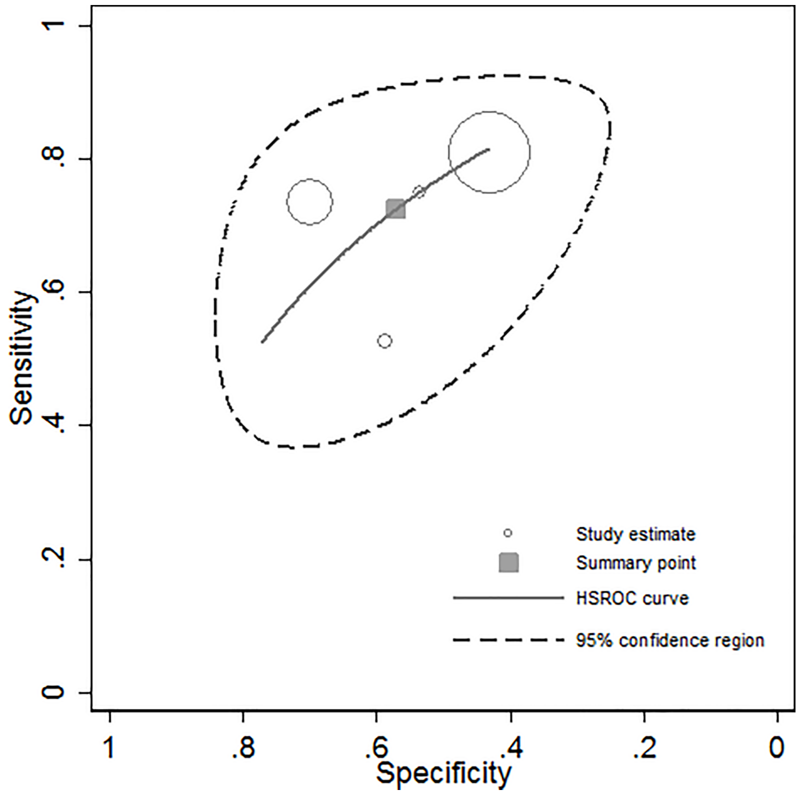

Fig 2. Performance of EZScan in the screening of T2DM: Meta-analysis using HSROC. Sensitivity = 72.0% (95%CI: 60.0%–83.0%); specificity = 56.0% (95%CI: 38.0%–74.0%); likelihood ratio positive = 1.68 (95%CI: 1.35–2.10); likelihood ratio negative = 0.48 (95%CI: 0.36–0.66); DOR = 3.49 (95%CI: 2.18–5.57). HSROC curve is shown only for sensitivities and specificities at least as large as the smallest study-specific estimates.

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Discussion

Summary of evidence

According to the results of this systematic review and meta-analysis, the performance of the EZSCAN in the detection of cases undiagnosed T2DM can be considered acceptable especially in the case of sensitivity, and even comparable to different well-known T2DM risk scores [35, 36]. To put in context our findings, the sensitivity of HbA1c, using a cut-off ≥6.5% (48 mmol/mol), for detecting undiagnosed diabetes was 52.8% using OGTT as the gold standard [37]. Thus, apparently, the EZSCAN might perform better that HbA1c although other studies are needed to corroborate this.

There are, however, some limitations that need to be highlighted. First, there is a risk of bias based on participant selection that can complicate extrapolation of results: many of the studies were performed in clinical context (i.e. clinical check-ups) instead of using population level assessments. Second, a high level of heterogeneity between studies was found (greater than 75%) in all estimations (i.e. sensitivity, specificity, etc). Since a small number of studies were included in the meta-analysis; results need to be cautiously interpreted despite of the fact that random effect models were used in calculations [38]. In addition, heterogeneity in results of the EZSCAN performance can be secondary to characteristics of the context and individuals: predictive values as well as likelihood ratios can depend on baseline risk of evaluated subjects. For example, the association of body mass index–one of the variables used in scoring individuals through EZSCAN–with the risk of diabetes may vary in different populations [39], and explain variability found in this report. Finally, although there is a suggested EZSCAN cut-off for defining T2DM in the population (50%), our results showed heterogeneity of this cut-off between studies and populations assessed: only two studies used the proposed 50% cut-off [32, 33], whereas the other studies were below that value. Thus, the device needs to be validated in different populations.

The principle of the EZSCAN, based on the evaluation of sudomotor function, relies on the assessment of chloride concentrations using reverse iontophoresis and chronoamperometry to detect insulin resistance and T2DM [18]. The EZSCAN has showed reproducible results in several conditions with low impact of usual physiological variations due to its focus on chloride concentrations, instead of sweat rates as used by other methods [40]. This device deliver results rapidly (i.e. in 2 to 3 minutes) and does not require invasive blood testing or fasting. Moreover, no safety problems have been reported during its use. Of note, although the EZSCAN has been designed to detect individuals with undiagnosed T2DM [18], some of the studies have focused on the ability of the device to detect impaired fasting glucose [17, 41, 42], dysglycemia [31, 33], metabolic syndrome [20], or even, complications related to T2DM [43, 44]. On the other hand, a relatively recent paper combined the performance of this device with conventional risk scores and reported limited improvement in the model given by the sum of EZSCAN plus risk score in Chinese population [31]. However, authors claimed that other studies are needed to determine the clinical relevance of EZSCAN in detecting cases of diabetes.

Public health relevance

Sensitivity and specificity estimates from this review may be used to better understand EZSCAN testing in real practice. For example, in a given setting with a prevalence of undiagnosed T2DM of 10% and assuming a cut-off value of 50% as suggested by the provider, if 1,000 individuals were screened using the EZSCAN, based on tool sensitivity, the device would detect 72 undiagnosed T2DM cases and 28 would be missing (false negatives). On the other hand, from the 900 individuals without the disease, 396 would be false positives and classified as having T2DM with the subsequent need of a confirmatory test. Thus, we would only need to perform 468 OGTT for those positive for EZSCAN, instead of the total population. If the prevalence were higher (i.e. 20% instead of 10%), of the 1,000 individuals, the device would detect 144 individuals based on its sensitivity, but 56 cases would be missing (false negatives). Of the 800 subjects without the disease, 352 would be false positives and classified as having T2DM with the need of a confirmatory test. Therefore, 496 OGTT would be needed but missing 56 cases as false negatives. On the other hand, summary estimates of the positive and negative likelihood ratios were very similar to values compatible with minimal change in the likelihood of disease. Thus, if positive and negative likelihood ratios of $>$10 and $<$0.1, respectively, were available, this would provide strong evidence to confirm and discard undiagnosed T2DM [45].

Using EZSCAN for detecting undiagnosed T2DM cases can have some advantages including the short time spent in conducting the test, the fact that fasting is not required, and the repeated used of the device can compensate its cost. However, some disadvantages also arise. Although, the EZSCAN can potentially reduce the resources implied in assessing populations for detecting T2DM cases, the number of false negatives (i.e. individuals with undiagnosed T2DM that are not detected by the device) increased when the prevalence of diabetes increased. On the other hand, literature suggested that EZSCAN cutoff should be estimated by each population instead of only using the cut-off given by the provider [31, 34, 46].

To our knowledge there is no information regarding the cost-effectiveness of the EZSCAN for detecting one undiagnosed case of T2DM in addition to the lack of data related to the potential performance for future risk of T2DM. Only one study has assessed the utility of this device longitudinally (2-year follow-up) but in a small sample [17]. In this study, the authors found an association between the EZSCAN score and T2DM progression although results needed further confirmation. Thus, the EZSCAN might have potential implications for T2DM prevention although population-based validation may be necessary to define appropriate cut-off for appropriate results interpretation.

Limitations

One of the limitations of this review is the representativeness of the results characterized by bias in participants' selection as well as the lack of a true gold standard in some of the studies (i.e. FG was used in one study instead of OGTT). In addition, characteristics of the study population were poorly reported and this is reflected in the quality assessment. As all the studies assessing EZSCAN were recently published (from 2010 and onwards); authors should have been utilized the Standards for Reporting Diagnostic Accuracy Studies (STARD) to guide their manuscripts' writing [47]. Future studies should follow these guidelines to guarantee an appropriate reporting of diagnostic studies.

Given the limited number of studies assessed, EZSCAN threshold was not meta-analyzed as the performance of the diagnostic test depends on the population in which the test is used. Thus, for our analyses, pooled sensitivity and specificity were calculated using the best cut-off reported by studies and not the same in all cases. In addition, there is limited data evaluating the potential impact of EZSCAN for undiagnosed T2DM at the population level. Future studies should be focused on population-based samples instead of referral health facilities, but also in different ethnic groups as only studies from China and India were used in this review. A study from Mexican population was also included in the meta-analysis, but the sample was biased and FG was used as gold standard [34]. Moreover, as the number of studies included in the analysis was small, publication bias was not assessed (usual tests for publication bias are underpowered when <10 studies are evaluated).

In summary, the sensitivity of the EZSCAN for undiagnosed T2DM screening seems to be acceptable but the evidence is limited because of the presence of participant selection bias in most of the included studies in the meta-analysis. The performance of the EZSCAN warrants confirmation in different populations, using the appropriate gold standard, and population-based samples. Moreover, adequate report of findings and longitudinal utility of the EZSCAN is also compulsory.

Supporting information

S1 Checklist. PRISMA Checklist information.
(DOC)

S1 Table. Search strategy and databases included for EZScan used in OVID.
(DOC)

S2 Table. Quality assessment of the studies included in the systematic review (QUADAS-2).
(DOC)

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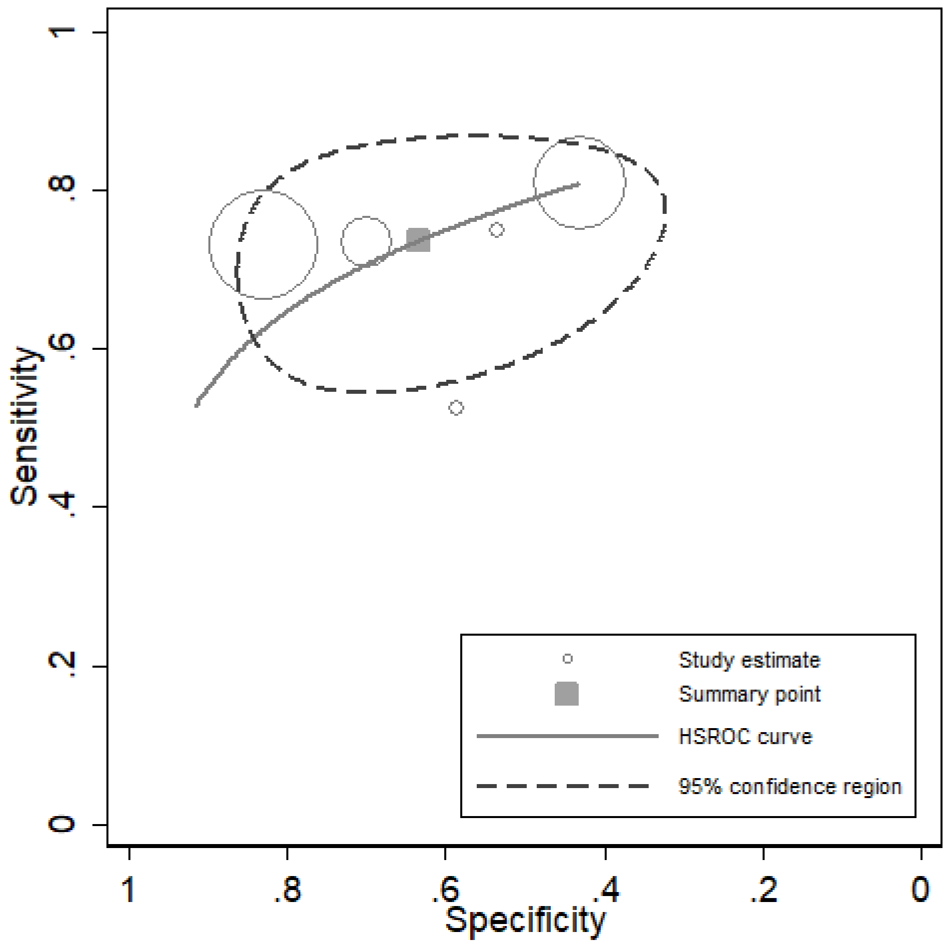
APPENDIX C

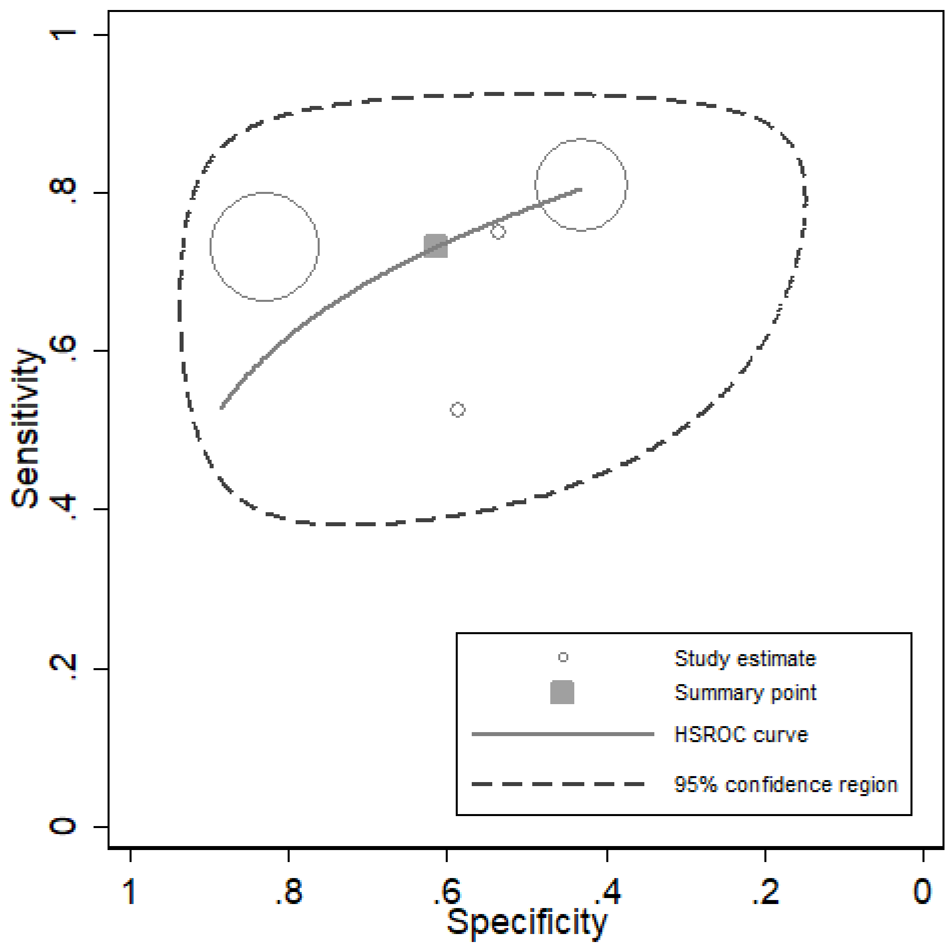
Figure: Performance of EZSCAN for undiagnosed T2DM: Updated meta-analysis using HS-ROC (using all studies, n = 5)



Sensitivity = 73.6% (95%CI: 65.6% - 80.3%); specificity = 63.2% (95%CI: 49.1% - 75.4%); likelihood ratio positive = 2.00 (95%CI: 1.40 – 2.86); likelihood ratio negative = 0.42 (95%CI: 0.31 – 0.57); DOR = 4.80 (95%CI: 2.60 – 8.87).

APPENDIX D

Figure: Performance of EZSCAN for undiagnosed T2DM: Updated meta-analysis using HS-ROC (using studies with OGTT, n = 4)



Sensitivity = 73.0% (95%CI: 62.8% - 81.3%); specificity = 61.3% (95%CI: 44.0% - 76.2%); likelihood ratio positive = 1.89 (95%CI: 1.24 – 2.88); likelihood ratio negative = 0.44 (95%CI: 0.30 – 0.65); DOR = 4.30 (95%CI: 2.02 – 9.15).

APPENDIX E:

Table: English version of the scoring of the FINDRISC and LA-FINDRISC for undiagnosed T2DM

	FINDRISC	LA-FINDRISC
Age:		
<45 years	0 points	0 points
45 – 54 years	2 points	2 points
55-64 years	3 points	3 points
65+ years	4 points	4 points
Body mass index:		
< 25 kg/m^2	0 points	0 points
Between 25 and < 30 kg/m^2	1 point	1 point
≥30 kg/m^2	3 points	3 points
Waist circumference:		
Men: <94 cm; women: <80 cm	0 points	0 points
Men: 94 – 102 cm; women: 80 – 88 cm	3 points	4 points
Men: >102 cm; women: >88 cm	4 points	
Physical activity (at least 30 min/day):		
Yes	0 points	0 points
No	2 points	2 points
Fruits and vegetables intake:		
Every day	0 points	0 points
Not every day	1 point	1 point
Regular medication for hypertension:		
No	0 points	0 points
Yes	2 points	2 points
History of high glucose levels:		
No	0 points	0 points
Yes	5 points	5 points
Diabetes in relatives:		
No	0 points	0 points
Yes, grandparents, cousins, uncle, aunt	3 points	3 points
Yes, parents, siblings, son, daughter	5 points	5 points

The difference between FINDRISC and LA-FINDRISC is based on score on waist circumference.

APPENDIX F:

Table: Spanish version of the questionnaire for participants enrolled in the study

Código del Participante:	☐☐☐ – ☐☐☐ – ☐☐	Código de trabajador:	☐☐

CRÓNICAS - PERU
CENTRO DE EXCELENCIA EN ENFERMEDADES CRÓNICAS

Evaluación de dos métodos alternativos para el diagnostico de diabetes: un estudio piloto para mejorar el tamizaje a nivel poblacional

Por favor, confirmar la siguiente información para asegurar el adecuado enrolamiento del participante

Por favor preséntese verbalmente antes de empezar:

"Buenos días / tardes, mi nombre es (decir su nombre y presentar su carnet). Soy personal de salud del Centro de Salud Global de la Universidad Peruana Cayetano Heredia. Estamos realizando un estudio de investigación sobre enfermedades crónicas como presión alta y diabetes. Nos gustaría hacerle una preguntas sobre sus datos generales y posteriormente le proporcionaré una hoja informativa sobre las razones del estudio, luego de eso Ud. decidirá si desea participar en el presente estudio"

Criterios de inclusión (1 = Si; 2 = No)		Respuesta
1	Edad entre 35 y 69 años	☐
2	Capaz de entender los procedimientos	☐
3	Capaz de dar consentimiento informado	☐
4	Residencia a tiempo completo en área de estudio (≥6 meses)	☐

Criterios de exclusión (1 = Si; 2 = No)		Respuesta
1	¿Está usted embarazada?	☐
2	¿Está usted postrado en cama?	☐
Exclusión para EZScan (1 = Si; 2 = No)		**Respuesta**
3	¿Usa usted un marcapasos cardiaco?	☐
4	¿Es usted alérgico al níquel?	☐
Exclusión para pupilometría (1 = Si; 2 = No)		**Respuesta**
5	¿Ha sido usted diagnosticado de enfermedad de Parkinson?	☐
6	¿Ha sido usted diagnosticado de enfermedad de Alzheimer?	☐
7	¿Ha sido usted diagnosticado de esclerosis múltiple?	☐
8	¿Presenta usted algún problema ocular severo (cataratas, glaucoma)?	☐
9	¿Presenta usted alguna lesión en la cornea?	☐

Código del Participante: ☐☐☐ – ☐☐☐ – ☐☐ **Código de trabajador:** ☐☐

Preguntas de supervisión (1 = Si; 2 = No)	Respuesta
¿El cuestionario está completo?	☐
¿Las medidas antropométricas fueron realizadas?	☐
¿Las medidas de presión arterial fueron realizadas?	☐
¿Las medidas de EZScan fueron realizadas?	☐
¿Las medidas de pupilometría fueron realizadas?	☐
¿Las medidas de biotensiómetro fueron realizadas?	☐
¿Las muestras de sangre fueron tomadas?	☐
¿Las muestras de sangre están completas?	☐

Código del Participante: ☐☐☐ – ☐☐☐ – ☐☐	**Código de trabajador:** ☐☐

Sección 1: Formato de Evaluación Demográfica

DNI del entrevistado	☐☐☐☐☐☐☐☐

Módulo: Lugar y fecha		**Respuestas**	
1	Identificación de entrevistadora (iniciales)		☐☐☐☐
2	Fecha (DD-MMM-20AA)		☐☐ - ☐☐☐ - ☐☐
3	Nombre de la villa donde se hace la entrevista	Nombre de villa	
		Vivienda #	

Módulo: Consentimiento		**Respuestas**	
4	Se ha leído el consentimiento al entrevistado	Si	1
		No	2 → **Si NO, leer consentimiento**
5	Se ha obtenido el consentimiento (escrito)	Si	1
		No	2 → **Si NO, terminar la entrevista**

Módulo: Información de contacto			
6	Apellidos completos		
7	Nombres completos		
8	Teléfonos de contacto	Celular	☐☐ – ☐☐☐☐☐☐☐☐☐
		Domicilio fijo:	☐☐☐☐☐☐
		Nombre (pariente)	
		Parentesco (1)	
		Celular (1)	☐☐ – ☐☐☐☐☐☐☐☐☐
		Domicilio Fijo (1)	☐☐☐☐☐☐
		Amigo o vecino	
		Celular (2)	☐☐ – ☐☐☐☐☐☐☐☐☐
		Domicilio fijo (2)	☐☐☐☐☐☐

La información contenida en esta sección debe guardarse separada del cuestionario ya que contiene información confidencial.

Código del Participante:	☐☐☐ – ☐☐☐ – ☐☐	Código de trabajador:	☐☐

Sección 2: Formato de Evaluación Socio-demográfica

Módulo: Información de entrevistadora		Respuesta
1	Identificación de entrevistadora (iniciales)	☐☐☐☐
2	Fecha (DD – MMM – AA)	☐☐ - ☐☐☐ - ☐☐

Módulo: Información demográfica		Respuesta	
3	Sexo (registre de acuerdo a lo observado)	1	Hombre
		2	Mujer
4	Fecha de nacimiento (DD – MMM - AA)	☐☐ - ☐☐☐ - ☐☐	
5	Su fecha de nacimiento es…	1	Exacta → **Pase a la pregunta 8**
		2	Aproximada
		99	No sabe / No responde
6	Años cumplidos a la fecha	☐☐ Años	
7	Su edad es…	1	Exacta
		2	Aproximada
		99	No sabe / No responde
8	¿Cuál es su estado civil?	1	Soltero
		2	Casado
		3	Conviviente
		4	Separado
		5	Divorciado
		6	Viudo(a)
		99	No sabe / No responde
9	¿Cuál es el nivel de educación más alto que ha alcanzado?	1	Sin nivel
		2	Inicial
		3	Primaria } ☐
		4	Secundaria } ☐
		5	Superior no universitaria } ☐
		6	Superior universitaria } ☐
		99	No sabe / No responde
10	Actualmente, ¿Está trabajando?	1	Si
		2	No
11	En el último mes, ¿a cuánto ascendió su ingreso (no incluya el apoyo de otros familiares)? Ingrese "99999" si no responde	☐☐☐☐☐ Soles	

Código del Participante:	☐☐☐ – ☐☐☐ – ☐☐	Código de trabajador:	☐☐

12	Tomando como referencia los últimos 12 meses: ¿Cuál fue su ingreso mensual (no incluya el apoyo de otros familiares)?	1	No recibe ingresos
		2	Hasta 100 soles
		3	Entre 101 y 450 soles
		4	Entre 451 y 750 soles
		5	Entre 751 y 1000 soles
		6	Entre 1001 y 1500 soles
		7	Más de 1500 soles
		99	No sabe / No responde
13	Tomando como referencia los últimos 12 meses: ¿Cuál fue el ingreso familiar mensual incluyendo el apoyo de todos los familiares? **Observación:** **Corroborar este dato con el de la pregunta anterior (este valor debe ser mayor o igual a la pregunta 12)**	1	Hasta 100 soles
		2	Entre 101 y 450 soles
		3	Entre 451 y 750 soles
		4	Entre 751 y 1000 soles
		5	Entre 1001 y 1500 soles
		6	Entre 1501 y 2000 soles
		7	Más de 2000 soles
		99	No sabe / No responde / Rehúsa responder
14	Usted se considera como: **(Leer todas las respuestas)**	1	Nativo Amazónico
		2	Nativo Quechua o Aymara
		3	Mestizo
		4	Afro-descendiente / Negro
		5	Caucásico / Blanco
		6	Asiático / Amarillo
		7	Otro
		99	No sabe / No responde
15	¿Ha vivido en este lugar toda su vida?	1	Si → **Pasar al siguiente módulo**
		2	No
16	¿Qué edad tenía cuando llegó a esta ciudad?	☐☐	Años

Módulo: Cobertura de salud		**Respuesta**	
17	Actualmente ¿se encuentra Ud. afiliado a algún sistema de salud?	1	Si
		2	No → **Pasar a la siguiente sección**
18	Especifique a cuál de estos sistemas de salud se encuentra afiliado. **(Acepte una o más alternativas)**	1	ESSALUD
		2	Seguro Integral de Salud
		3	Seguro privado / Entidad prestadora de salud
		4	Otro seguro
		99	No sabe / No responde

Código del Participante:	☐☐☐ – ☐☐☐ – ☐☐	Código de trabajador:	☐☐

Sección 3: Formato de Evaluación de la Vivienda

Módulo: Características de la familia		Respuesta
1	¿Cuántas personas en total, incluyéndolo a Ud., viven en su casa?	☐☐ Personas
2	¿Cuántas de estas personas son mayores de 18 años? (inclúyase Ud.)	☐☐ Personas
3	¿Cuántas familias que cocinan sus propios alimentos viven en su vivienda?	☐☐ Número de familias
4	¿Cuántos ambientes de su vivienda se usan solo para dormir?	☐☐ Número de ambientes

Módulo: Posesiones en la vivienda			
5	Tiene en su hogar… **(Leer las opciones, verificar si funcionan y marcar todas las que apliquen)**	1	Cocina a gas
		2	Inodoro con desagüe
		3	Radio / equipo de sonido
		4	Horno microondas
		5	Licuadora
		6	Plancha
		7	TV a color
		8	Refrigerador
		9	Lavadora
		10	Computadora
		11	Teléfono fijo
		12	Celular
		13	Conexión a cable
		14	Conexión a Internet
		15	Bicicleta para adultos
		16	Motocicleta
		17	Carro
		99	Rehúsa responder

Módulo: Facilidades en la vivienda		Respuesta	
6	¿Cuál es la fuente principal de abastecimiento de agua que utilizan en su hogar? **(Leer todas las opciones y marcar la que aplica)**	1	Caño dentro de la vivienda
		2	Pozo en la casa o lote
		3	Caño o pilón de uso público
		4	Pozo público
		5	Manantial
		6	Río/acequia
		7	Camión, tanque o aguatero
		8	Otro: ______________________________
		99	Rehúsa responder

Código del Participante: ☐☐☐ – ☐☐☐ – ☐☐ **Código de trabajador:** ☐☐

7	¿Cuál es el material predominante de los pisos de su vivienda?	1	Piso natural: tierra o arena
		2	Piso rústico: entablado
		3	Piso de cemento no acabado
		4	Parquet, vinílicos, losetas, cemento
		99	Rehúsa responder
8	¿Cuál es el material predominante en los techos de su vivienda?	1	Esteras, paja, hojas de palmera
		2	Calamina, madera, caña, fibra de cemento
		3	Tejas
		4	Concreto armado o cemento
		99	Rehúsa responder
9	Mayormente, ¿qué tipo de combustible utiliza para cocinar?	1	Leña
		2	Estiércol, bosta, heces, etc.
		3	Carbón
		4	Kerosene
		5	Gas propano
		6	Electricidad
		7	Otro
		99	Rehúsa responder

Código del Participante: ☐☐☐ – ☐☐☐ – ☐☐ **Código de trabajador:** ☐☐

Sección 4: Formato de Evaluación de Estilos de Vida (LAF)

Módulo: Consumo de tabaco		Respuesta	
1	Actualmente…	1	No fumo
		2	Fumo ocasionalmente
		3	Fumo diariamente (al menos uno al día)
		99	Rehúsa responder
2	¿Cuál describe mejor su historia de consumo de tabaco?	1	Nunca fumó → **Pase al siguiente modulo**
		2	Fumo anteriormente
		3	Fuma actualmente
		99	Rehúsa responder
3	¿Qué edad tenía cuando comenzó a fumar o probó cigarrillos por primera vez en su vida?	☐☐	Años (99 = No sabe/no recuerda)
4	¿Cuándo fue la última vez que fumó un cigarrillo?	1	Menos de 1 mes
		2	Entre 1 y 6 meses → **Pase al siguiente modulo**
		3	Entre 6 y 12 meses → **Pase al siguiente modulo**
		4	Un año y más → **Pase al siguiente modulo**
		99	No sabe / No responde
5	¿Cuántos cigarrillos fumó en total en los <u>últimos treinta días</u>?	☐☐☐	Número de cigarrillos (999 = No sabe/no responde)

Módulo: Uso de alcohol		Respuesta	
6	¿Con qué frecuencia consume alguna bebida alcohólica?	1	Nunca → **Pase a la pregunta 16**
		2	Una o menos veces al mes
		3	De 2 a 4 veces al mes
		4	De 2 a 3 veces a la semana
		5	4 o más veces a la semana
		99	Rehúsa responder
7	¿Cuántas botellas de cerveza o su equivalente en otras bebidas puede beber en un día normal de consumo?	1	1 ó 2
		2	3 ó 4
		3	5 ó 6
		4	7 a 9
		5	10 ó mas
		99	Rehúsa responder
8	¿Con qué frecuencia toma 6 o más botellas de cerveza o su equivalente en bebidas alcohólicas en una misma ocasión de consumo?	1	Nunca
		2	Menos de 1 vez al mes
		3	Mensualmente
		4	Semanalmente
		5	A diario o casi a diario
		99	Rehúsa responder

Código del Participante:	☐☐☐ – ☐☐☐ – ☐☐	Código de trabajador:	☐☐

9	¿Con qué frecuencia en el curso del último año ha sido incapaz de parar de beber una vez que había empezado?	1	Nunca
		2	Menos de 1 vez al mes
		3	Mensualmente
		4	Semanalmente
		5	A diario o casi a diario
		99	Rehúsa responder
10	¿Con qué frecuencia en el curso del último año no pudo hacer lo que otros esperaban de usted porque había bebido?	1	Nunca
		2	Menos de 1 vez al mes
		3	Mensualmente
		4	Semanalmente
		5	A diario o casi a diario
		99	Rehúsa responder
11	¿Con qué frecuencia en el curso del último año ha necesitado de beber en ayunas para recuperarse después de haber bebido mucho el día anterior?	1	Nunca
		2	Menos de 1 vez al mes
		3	Mensualmente
		4	Semanalmente
		5	A diario o casi a diario
		99	Rehúsa responder
12	¿Con qué frecuencia en el curso del último año ha tenido remordimientos o sentimientos de culpa después de haber bebido?	1	Nunca
		2	Menos de 1 vez al mes
		3	Mensualmente
		4	Semanalmente
		5	A diario o casi a diario
		99	Rehúsa responder
13	¿Con qué frecuencia en el curso del último año no ha podido recordar lo que sucedió la noche anterior porque había estado bebiendo?	1	Nunca
		2	Menos de 1 vez al mes
		3	Mensualmente
		4	Semanalmente
		5	A diario o casi a diario
		99	Rehúsa responder
14	¿Con qué frecuencia tiene Ud. resaca?	1	Nunca
		2	Menos de 1 vez al mes
		3	Mensualmente
		4	Semanalmente
		5	A diario o casi a diario
		99	Rehúsa responder
15	Si tuviera que calificar su consumo de alcohol, Ud. diría que mayormente es:	1	Acompañando las comidas
		2	Mayoría de fines de semana o vacaciones
		3	Momentos o motivos ocasionales
		99	Rehúsa responder

Código del Participante:	☐☐☐ – ☐☐☐ – ☐☐	Código de trabajador:	☐☐

16	¿Usted o alguna persona han resultado heridos porque usted había estado bebiendo?	1	No
		2	Sí, pero no durante el último año
		3	Sí, durante el último año
		99	Rehúsa responder
17	¿Algún familiar, amigo, médico o profesional sanitario han mostrado preocupación por su consumo de bebidas alcohólicas o le han indicado que deje de beber?	1	No
		2	Sí, pero no durante el último año
		3	Sí, durante el último año
		99	Rehúsa responder

Módulo: Actividad física		Respuesta	
18	¿Considera usted que es físicamente activo?	1	Si
		2	No
		99	Rehúsa responder
19	¿Comparando su actividad física con otros sujetos de la misma edad, considera usted que físicamente activo?	1	Si
		2	No
		99	Rehúsa responder
20	¿Realiza habitualmente al menos 30 minutos de actividad física, en el trabajo y/o en su tiempo libre?	1	Si
		2	No
		99	Rehúsa responder
21	¿Cuánto tiempo (en horas) diría usted que gasta usualmente sentado o reclinado en un día típico?	☐☐	Número de horas por día
		99	No sabe/ Rehúsa responder

Módulo: Actividad física intensas		Respuesta	
LEA: Piense en todas las actividades físicas **intensas** que usted realizó en los **últimos 7 días**. Las actividades físicas **intensas** se refieren a aquellas que implican un esfuerzo físico intenso y que lo hacen respirar mucho más intensamente que lo normal. Piense solo en aquellas actividades físicas que realizó durante por lo menos **10 minutos** seguidos.			
22	Durante los últimos 7 días, ¿en cuántos días realizó actividades físicas **intensas** tales como levantar pesos pesados, cavar, hacer ejercicios aeróbicos o andar rápido en bicicleta?	☐☐	Días por semana→ **Si 00 pase a p24**
		99	No sabe/ Rehúsa responder → **Pase a p24**
23	Habitualmente, ¿cuánto tiempo en total dedicó a una actividad física **intensa** en uno de esos días?	☐☐ : ☐☐	Tiempo (HH:MM) por día
		99	No sabe/ Rehúsa responder

Módulo: Actividad física moderadas		Respuesta	
LEA: Piense en todas las actividades físicas **moderadas** que usted realizó en los **últimos 7 días**. Las actividades físicas **moderadas** son aquellas que requieren un esfuerzo físico moderado que lo hacen respirar algo más intensamente que lo normal. Piense solo en aquellas actividades físicas que realizó durante por lo menos **10 minutos** seguidos.			
24	Durante los últimos 7 días, ¿en cuántos días realizó actividades físicas **moderadas** como transportar pesos livianos, andar en bicicleta a velocidad regular, subir cerros? No incluya caminar	☐☐	Días por semana→ **Si 00 pase a p26**
		99	No sabe/ Rehúsa responder → **Pase a p26**

Código del Participante:	☐☐☐ – ☐☐☐ – ☐☐	Código de trabajador:	☐☐

25	Habitualmente, ¿cuánto tiempo en total dedicó a una actividad física **moderada** en uno de esos días?	☐☐ : ☐☐	Tiempo (HH:MM) por día
		99	No sabe/ Rehúsa responder

Módulo: Actividad física leves		**Respuesta**	
LEA: Piense en el tiempo que usted dedicó a **caminar** en los **últimos 7 días**. Esto incluye caminar en el trabajo o en la casa, para trasladarse de un lugar a otro, o cualquier otra caminata que usted podría hacer solamente para la recreación, el deporte, el ejercicio o el ocio.			
26	Durante los **últimos 7 días**, ¿en cuántos días caminó durante por lo menos **10 minutos** seguidos?	☐☐	Días por semana→ **Si 00 pase a p28**
		99	No sabe/ Rehúsa responder → **Pase a p28**
27	Habitualmente, ¿cuánto tiempo en total dedicó a caminar en uno de esos días?	☐☐ : ☐☐	Tiempo (HH:MM) por día
		99	No sabe/ Rehúsa responder

Módulo: Ausencia de actividad física		**Respuesta**	
28	Durante los **últimos 7 días**, de lunes a viernes, ¿Cuánto tiempo pasó sentado viendo TV?	☐☐	Horas por día (Colocar **00** si es <1 hora)
		99	No sabe/ Rehúsa responder
29	Durante los **últimos 7 días**, en el fin de semana, ¿Cuánto tiempo pasó sentado viendo TV?	☐☐	Horas por día (Colocar **00** si es <1 hora)
		99	No sabe/ Rehúsa responder

Módulo: Patrones de dieta		**Respuesta**	
Instrucciones: Pregunte al participante que tan frecuentemente consume comida de cada una de las siguientes categorías. Coloque en el recuadro según la frecuencia: **1** = Nunca; **2** = 1 a 3 veces/mes **3** = 1 vez por semana; **4** = 2 a 4 veces por semana **5** = 5 a 6 veces por semana; **6** = 1 vez por día **7** = Mas de 1 vez por día			
Durante el último mes, en promedio con qué frecuencia consumió:		**Frecuencia**	**Número de veces**
30	**Vegetales verdes:** lechuga, espinaca, espárragos, brócoli, etc.	☐ →Si 7 →	☐☐
31	**Vegetales crudos (no verdes):** zanahorias, tomates, etc.	☐ →Si 7 →	☐☐
32	**Vegetales cocidos (no verdes):** zanahorias, tomates, etc.	☐ →Si 7 →	☐☐
33	**Frutas:** plátanos, naranjas, manzanas, fresas, frutas secas, etc.	☐ →Si 7 →	☐☐
34	**Jugos y néctares artificiales:** Frugo's, Pulp, Cifrut, Aquarius, etc.	☐ →Si 7 →	☐☐
35	**Bebidas gaseosas:** Coca Cola, Inka Cola, Fanta, etc.	☐ →Si 7 →	☐☐
36	**Bebidas rehidratantes:** Sporade, Gatorade, etc.	☐ →Si 7 →	☐☐
37	**Té y otras infusiones** (hierbaluisa, anis, etc.)	☐ →Si 7 →	☐☐
38	**Café**	☐ →Si 7 →	☐☐
39	**Refrescos:** limonada, agua de manzana, etc.	☐ →Si 7 →	☐☐

Código del Participante:	☐☐☐ – ☐☐☐ – ☐☐	Código de trabajador:	☐☐

Durante el último mes, en promedio con qué frecuencia añadió azúcar a alguna bebida:					
		# cucharaditas	**Al ras**	**Normal**	**Colmada**
40	**Té y otras infusiones** (hierbaluisa, anis, etc.)	☐☐	☐	☐	☐
41	**Café**	☐☐	☐	☐	☐
42	**Refrescos:** limonada, agua de manzana, etc.	☐☐	☐	☐	☐

Módulo: Horas de sueño

43	¿Cómo promedio, en el último año, cuántas horas durmió en un día (incluyendo siestas)?	☐☐	Número de horas (99 = No sabe/no recuerda)
44	Durante el último mes, ¿ha tenido dificultades para poder dormir?	1	Casi nunca
		2	A veces
		3	Con frecuencia
45	Durante el último mes, ¿qué tan frecuentemente se despierta durante la noche?	1	Casi nunca
		2	A veces
		3	Con frecuencia

Módulo: Escala de Epworth (Versión peruana modificada)

46	¿Usted maneja vehículos motorizados (auto, camioneta, ómnibus, micro, combi, etc.)?	1	Si
		2	No

Instrucciones: ¿Qué tan probable es que usted cabecee o se quede dormido en las siguientes situaciones? No se refiere a sentirse cansado debido a actividad física. Aunque no haya realizado últimamente las siguientes situaciones descritas, considere como le habrían afectado. Use la siguiente escala y marque la opción más apropiada para cada situación:

0 = Nunca cabecearía,
1 = Poca probabilidad de cabecear,
2 = Moderada probabilidad de cabecear,
3 = Alta probabilidad de cabecear.

47	Sentado leyendo	☐
48	Viendo televisión	☐
49	Sentado (por ejemplo en el teatro, en una reunión, en el cine, en una conferencia, o en misa o culto)	☐
50	Como pasajero en un automóvil, ómnibus, micro o combi durante una hora o menos de recorrido	☐
51	Recostado en la tarde si las circunstancias lo permiten	☐
52	Sentado conversando con alguien	☐
53	Sentado luego del almuerzo y sin haber bebido alcohol	☐
54	Conduciendo el automóvil cuando se detiene algunos minutos por razones de tráfico	☐
55	Parado y apoyándose o no en una pared o mueble	☐

Módulo: Ronquidos y apnea

56	¿Alguna vez le han dicho que Ud. ha roncado (ahora o en cualquier momento en el pasado)?	1	Si
		2	No → **Pase a la pregunta 59**
		99	No sabe /rehúsa responder

Código del Participante: ☐☐☐ – ☐☐☐ – ☐☐ **Código de trabajador:** ☐☐

57	¿Qué tan frecuente Ud. ronca? (Marcar solo una respuesta)	1	No he roncado nunca
		2	Rara vez – Menos de 1 noche por semana
		3	Algunas veces – 1 a 2 noches por semana
		4	Frecuentemente – 3 a 5 noches por semana
		5	Siempre o casi siempre
		99	No sabe /rehúsa responder
58	¿Qué tan fuerte es su ronquido? (Marcar solo una respuesta)	1	Un poco más fuerte que respiración profunda
		2	Tan fuerte como murmurar o hablar
		3	Más fuerte que hablar
		4	Muy fuerte – se escucha tras una puerta cerrada
		99	No sabe /rehúsa responder
59	Basado en lo que Ud. ha notado o los otros miembros de su vivienda le han dicho, ¿hay veces que su respiración se detiene mientras Ud. duerme?	1	Si
		2	No
		99	No sabe /rehúsa responder
60	¿Algún miembro de su familia le ha dicho que durante su sueño Ud. suena como si se estuviera ahogando?	1	Si
		2	No
		99	No sabe /rehúsa responder

Módulo: Calidad de sueño

Lea: ***“****Las siguientes preguntas solo tienen que ver son sus hábitos de sueño durante el último mes. En sus respuestas debe reflejar cual ha sido su comportamiento durante la mayoría de los días y noches del pasado mes“.*

61	Durante el último mes, ¿cuál ha sido, normalmente, su hora de acostarse?	☐☐ : ☐☐	(Colocar en sistema de 24 horas)
62	¿Cuánto tiempo habrá tardado en dormirse, normalmente, las noches del último mes?	1	Menos de 15 minutos
		2	Entre 16 y 30 minutos
		3	Entre 31 y 60 minutos
		4	Más de 60 minutos
63	Durante el último mes, ¿a qué hora se ha levantado habitualmente por la mañana?	☐☐ : ☐☐	(Colocar en sistema de 24 horas)
64	¿Cuántas horas calcula que habrá dormido verdaderamente cada noche durante el último mes?	☐☐	Número de horas

65 **Lea:** *“Durante el último mes, ¿Cuántas veces ha tenido usted problemas para dormir a causa de…”*

Marque según corresponda:
0 = Ninguna vez en el último mes
1 = Menos de una vez a la semana
2 = Una o dos veces a la semana
3 = Tres o más veces a la semana

a.	No poder conciliar el sueño en la primera media hora	☐
b.	Despertarse durante la noche o de madrugada	☐
c.	Tener que levantarse para ir al servicio	☐
d.	No poder respirar bien	☐
e.	Toser o roncar ruidosamente	☐
f.	Sentir frio	☐

	g. Sentir demasiado calor		☐
	h. Tener pesadillas o malos sueños		☐
	i. Sufrir dolores		☐
	j. Otras razones (describir):		☐
66	Durante el último mes, ¿Cómo valoraría en conjunto, la calidad de su sueño?	1	Muy buena
		2	Bastante buena
		3	Bastante mala
		4	Muy mala
67	Durante el último mes, ¿Cuántas veces habrá tomado medicinas (por su cuenta o recetadas por el médico) para dormir?	1	Ninguna vez en el último mes
		2	Menos de una vez a la semana
		3	Una o dos veces a la semana
		4	Tres o más veces a la semana
68	Durante el último mes, ¿Cuántas veces ha sentido somnolencia mientras conducía, comía o desarrollaba alguna otra actividad?	1	Ninguna vez en el último mes
		2	Menos de una vez a la semana
		3	Una o dos veces a la semana
		4	Tres o más veces a la semana
69	Durante el último mes, ¿Ha representado para usted mucho problema el tener ánimos para realizar alguna de las actividades detalladas en la pregunta anterior?	1	Ningún problema
		2	Solo un leve problema
		3	Un problema
		4	Un grave problema
70	¿Duerme usted solo o acompañado?	1	Solo
		2	Con alguien en otra habitación
		3	En la misma habitación, pero en otra cama
		4	En la misma cama

Código del Participante:	☐☐☐ – ☐☐☐ – ☐☐	Código de trabajador:	☐☐

Sección 5: Formato de Evaluación de Salud mental (MHF)

Módulo: Síntomas depresivos		Respuesta
Instrucciones: Escoja una de las opciones de acuerdo a las respuestas del participante: 0 = Nunca 1 = Varios días 2 = Más de la mitad de los días 3 = Casi todos los días		
Pregunta: Durante las <u>últimas 2 semanas</u>, ¿con qué frecuencia le han molestado los siguientes problemas?		
1	Tener poco interés o placer en hacer las cosas	☐
2	Sentirse desanimado/a, deprimido/a, triste o sin esperanza	☐
3	Problemas en dormirse o mantenerse dormido/a, o en dormir demasiado	☐
4	Sentirse cansado/a o tener poca energía	☐
5	Tener poco apetito o comer en exceso	☐
6	Sentirse mal acerca de sí mismo/a – o sentir que es un/una fracasado/a o que se ha fallado a si mismo/a o a su familia	☐
7	Dificultad para poner atención, concentrarse en cosas tales como leer el periódico o ver televisión	☐
8	Moverse o hablar tan despacio que otras personas lo pueden haber notado – o lo contrario: estar tan inquieto/a o intranquilo/a que se ha estado moviendo mucho más de lo normal	☐
9	Pensamientos de que sería mejor estar muerto/a o que quisiera hacerse daño de alguna forma	☐

Módulo: Ansiedad		Respuesta	
Lea: "*A continuación me gustaría hacerle algunas preguntas para saber si ha tenido alguno de los siguientes síntomas en las <u>últimas dos semanas</u>.*"			
10	¿Se ha sentido muy excitado, nervioso o tensión?	1	Si
		2	No
11	¿Ha estado muy preocupado por algo?	1	Si
		2	No
12	¿Se ha sentido muy irritable?	1	Si
		2	No
13	¿Ha tenido dificultad para relajarse?	1	Si
		2	No
14	¿Ha dormido mal, ha tenido dificultades para dormir?	1	Si
		2	No
15	¿Ha tenido dolores de cabeza o nuca?	1	Si
		2	No
16	¿Ha tenido alguno de los siguientes síntomas: temblores, hormigueos, mareos, sudores, diarrea?	1	Si
		2	No
17	¿Ha estado preocupado por su salud?	1	Si
		2	No

Código del Participante:	☐☐☐ – ☐☐☐ – ☐☐	Código de trabajador:	☐☐

18	¿Ha tenido alguna dificultad para conciliar el sueño, para quedarse dormido?	1	Si
		2	No

Módulo: Calidad de vida		**Respuesta**	
Instrucciones: Marque la respuesta que mejor describe su estado de salud en el <u>día de hoy</u>.			
19	Movilidad	1	No tengo problemas para caminar
		2	Tengo algunos problemas para caminar
		3	Tengo que estar en cama
20	Cuidado personal	1	No tengo problemas con mi cuidado personal
		2	Tengo algunos problemas para lavarme o vestirme solo
		3	Soy incapaz de lavarme o vestirme solo
21	Actividades habituales (por ejemplo, estudiar, hacer tareas domésticas, actividades familiares o realizadas durante el tiempo libre)	1	No tengo problemas para realizar mis actividades
		2	Tengo algunos problemas para realizar mis actividades
		3	Soy incapaz de realizar mis actividades habituales
22	Dolor o malestar	1	No tengo dolor ni malestar
		2	Tengo dolor o malestar moderado
		3	Tengo mucho dolor o malestar
23	Ansiedad o depresión	1	No estoy ansioso ni deprimido
		2	Estoy moderadamente ansioso o deprimido
		3	Estoy muy ansioso o deprimido

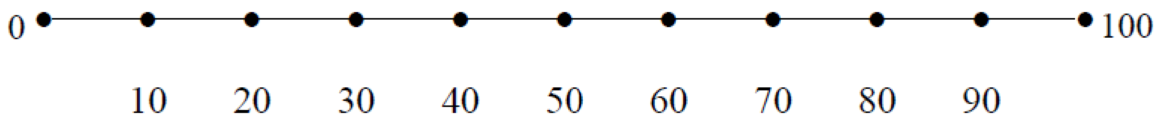
Módulo: Termómetro de Autovaloración del Estado de Salud (Calidad de vida)

Para ayudar a la gente a describir lo bueno o malo que es su estado de salud hemos dibujado una escala parecida a un termómetro en el cual se marca con un 100 el mejor estado de salud que pueda imaginarse y con un 0 el peor estado de salud que pueda imaginarse. Nos gustaría que nos indicara en esta escala, en su opinión, lo bueno o malo que es su estado de salud el día de hoy.

Instrucciones: Muestre la hoja al participante y coloque el valor del termómetro en el cuadro provisto (al lado de "Su estado de salud hoy").

Peor estado de salud que pueda imaginarse

Mejor estado de salud que pueda imaginarse



<u>Su estado de salud **HOY**:</u> ☐☐☐

Módulo: Situaciones importantes

Instrucciones: En el último año, alguna vez experimentó algo de lo siguiente:

1 = Si 2 = No 9 = No responde

24	Separación / divorcio	☐
25	Pérdida del empleo / jubilación	☐
26	Pérdidas en su negocio	☐

Código del Participante:	☐☐☐ – ☐☐☐ – ☐☐	Código de trabajador:	☐☐

27	Conflicto familiar importante	☐
28	Lesión o enfermedad importante	☐
29	Muerte de cónyuge (esposo/a)	☐
30	Muerte de hijo o algún familiar cercano	☐

	Módulo: Estrés percibido	**Respuesta**
	Lea: *“Las preguntas a continuación se refieren a los sentimientos y pensamientos que ha tenido durante el último mes.”* Instrucciones: Marque según corresponda: **1** = Nunca **2** = Casi nunca **3** = De vez en cuando **4** = Frecuentemente **5** = Casi siempre	
31	En el último mes, ¿te has sentido molesto a causa de alguna situación inesperada?	☐
32	En el último mes, ¿te has sentido incapaz de controlar hechos importantes en tu vida?	☐
33	En el último mes, ¿te has sentido continuamente tenso?	☐
34	En el último mes, ¿resolviste de manera exitosa las discusiones desagradables en tu vida?	☐
35	En el último mes, ¿sentiste que enfrentaste exitosamente los cambios que estaban ocurriendo en tu vida?	☐
36	En el último mes, ¿confiaste en tu capacidad para manejar tus problemas personales?	☐
37	En el último mes, ¿sentiste que las cosas te estaban resultando como tú querías?	☐
38	En el último mes, ¿encontraste que no podías resolver todas las situaciones que tenias que enfrentar?	☐
39	En el último mes, ¿has podido controlar los hechos desagradables de tu vida?	☐
40	En el último mes, ¿sentiste que estabas colapsado con las situaciones que te ocurrieron?	☐
41	En el último mes, ¿te has sentido molesto por situaciones que estaban fuera de tu control?	☐
42	En el último mes, ¿te has encontrado pensando en las situaciones que tienes que resolver?	☐
43	En el último mes, ¿has sido capaz de manejar tu tiempo según tus propias necesidades?	☐
44	En el último mes, ¿sentiste que los problemas se te iban acumulando?	☐

	Módulo: Soporte social	**Respuesta**
	Instrucciones: En la siguiente lista se muestran algunas cosas que otras personas hacen por nosotros o nos proporcionan. Elija para cada una la respuesta que mejor refleje su situación, según los siguientes criterios: **1** = Mucho menos de lo que deseo **2** = Menos de lo que deseo **3** = Ni mucho ni poco **4** = Casi como deseo **5** = Tanto como deseo	
45	Recibo visitas de mis amigos y familiares	☐
46	Recibo ayuda en asuntos relacionados con mi casa	☐
47	Recibo elogios y reconocimientos cuando hago bien mi trabajo	☐
48	Cuento con personas que se preocupan de lo que me sucede	☐
49	Recibo amor y afecto	☐
50	Tengo la posibilidad de hablar con alguien de mis problemas en el trabajo o en la casa	☐

Código del Participante:	☐☐☐ – ☐☐☐ – ☐☐	Código de trabajador:	☐☐

51	Tengo la posibilidad de hablar con alguien de mis problemas personales y familiares	☐
52	Tengo la posibilidad de hablar con alguien de mis problemas económicos	☐
53	Recibo invitaciones para distraerme y salir con otras personas	☐
54	Recibo consejos útiles cuando me ocurre algún acontecimiento importante en mi vida	☐
55	Recibo ayuda cuando estoy enfermo en la cama	☐

Módulo: Parkinsonismo		**Respuesta**	
Instrucciones: Estamos intentando evaluar la utilidad de este cuestionario. Quisiéramos que nos ayudara contestando a las siguientes preguntas:			
56	¿Tiene Ud. problemas para levantarse de una silla?	1	Si
		2	No
57	¿Ha notado si su escritura se ha hecho más pequeña que antes?	1	Si
		2	No
58	¿Le han comentado sobre si el volumen de su voz es menos potente que antes?	1	Si
		2	No
59	¿Ha notado que su equilibrio está alterado?	1	Si
		2	No
60	¿Ha notado que los pies se le quedan pegados al suelo al cruzar el umbral de las puertas?	1	Si
		2	No
61	¿Le parece que su cara es ahora menos expresiva?	1	Si
		2	No
62	¿Le tiemblan los brazos y piernas?	1	Si
		2	No
63	¿Tiene dificultad para abrocharse los botones?	1	Si
		2	No
64	¿Arrastra los pies y da pasitos cortos al andar?	1	Si
		2	No

Código del Participante:	☐☐☐ – ☐☐☐ – ☐☐	Código de trabajador:	☐☐

Sección 6: Formato de Antecedentes Cardiovascular (HAF)

Módulo: Antecedentes personales			**Respuesta**		
1	¿Ha sufrido o le han dicho que tiene alguna vez de estas enfermedades? (Por algún profesional de salud)		**Fue diagnosticado:** **1** = Si **2** = No **9** = NS/NR	**Quién fue:** **1** = Médico **2** = Enfermera **3** = Farmacéutico **4** = Otro **9** = NS/NR	**# años desde el diagnóstico** (00 si es < 1 año)
	(Leer las opciones y marcar todas las que aplican)	Presión arterial alta	☐	☐	☐☐
		Derrame cerebral	☐	☐	☐☐
		Infarto (ataque) cardiaco	☐	☐	☐☐
		Insuficiencia cardiaca	☐	☐	☐☐
		Colesterol alto	☐	☐	☐☐
		Diabetes	☐	☐	☐☐
		Cáncer	☐	☐	☐☐
		Especifique el tipo de cáncer:			

Módulo: Diagnóstico y tratamiento de diabetes		**Respuesta**	
2	¿Alguna vez algún doctor (o cualquier otro profesional de salud) le ha medido la glucosa (azúcar) en la sangre?	1	Si
		2	No → **Pasar a la pregunta 5**
3	¿Alguna vez le han encontrado niveles de glucosa (azúcar en sangre) altos (en un examen médico, durante alguna enfermedad, o durante el embarazo)?	1	Si
		2	No
4	¿Cuándo fue la última vez que le midieron la glucosa (azúcar) en la sangre?	1	Menos de 1 año
		2	Entre 1 y 2 años
		3	Más de 2 años
		9	No recuerda
5	En estos momentos, ¿algún médico le ha indicado algún tratamiento para controlar el azúcar en su sangre (diabetes)?	1	Si
		2	No → **Pasar al siguiente módulo**
6	¿Tiene Ud. indicado algún tratamiento con medicamentos para controlar la diabetes?	1	Si
		2	No → **Pasar al siguiente módulo**
7	Enumere otros medicamentos que está actualmente tomando para controlar la diabetes. **Por favor, pida el medicamento y copie el nombre y presentación.**	1	
		2	
		3	
		4	
		5	
8	¿Toma los medicamentos para controlar la diabetes en el **horario** establecido?	1	Siempre
		2	Casi siempre
		3	A veces
		4	Casi nunca
		5	Nunca

Código del Participante:	☐☐☐ – ☐☐☐ – ☐☐	Código de trabajador:	☐☐

9	¿Toma los medicamentos para controlar la diabetes en las **dosis** indicadas?	1	Siempre
		2	Casi siempre
		3	A veces
		4	Casi nunca
		5	Nunca
10	En el último año, ¿ha sido hospitalizado debido a su diabetes?	1	Si
		2	No → **Pasar a la pregunta 12**
11	En el último año, ¿Cuántas veces ha sido hospitalizado debido a su diabetes?	☐☐	Número de veces
12	¿Algún médico le ha dicho que presenta complicaciones debido a la diabetes?	1	Si
		2	No → **Pasar al siguiente módulo**
13	¿En qué parte del cuerpo le han dicho que presenta dichas complicaciones? (**Marque todas las que aplican**)	1	Ojos (retina)
		2	Cardiaca (presión arterial o corazón)
		3	Renal (riñones)
		4	Pies (neuropatía)
		5	Otros (***especifique***)

	Módulo: Antecedentes familiares de diabetes	**Respuesta**	
14	¿Alguno de los miembros de su familia ha sido diagnosticado con diabetes?	1	Si
		2	No → **Pasar al siguiente módulo**
15	¿Quién de su familia fue diagnosticado con diabetes? (Solo considere aquellos familiares de sangre, y marque todas las que aplican)	Padre	☐
		Madre	☐
		Hermano o hermana	☐
		Hijo o hija	☐
		Abuelos	☐
		Tíos o tías	☐
		Primos de primer grado	☐

	Módulo: Otros antecedentes de importancia	**Respuesta**		
16	Enumere todos los medicamentos que está actualmente tomando al menos una vez por semana durante el último mes para controlar	**TOMA PARA…**	1 = Si 2 = No 9 = NR	**MEDICAMENTO** Indicar nombre
		Presión arterial alta	☐	
		Derrame cerebral	☐	
		Infarto(ataque) cardiaco	☐	
		Insuficiencia cardiaca	☐	
		Colesterol alto	☐	
		Arritmia cardiaca	☐	

Código del Participante:	☐☐☐ – ☐☐☐ – ☐☐	Código de trabajador:	☐☐

17	Enumere otros medicamentos que está actualmente tomando al menos una vez por semana en el último mes. **Por favor, pida el medicamento y copie el nombre y presentación.**	1	
		2	
		3	
		4	

Módulo: Accidente cerebro-vascular		**Respuesta**
Instrucciones: Escribir en el recuadro según corresponda: **1** = Si **2** = No **9** = No responde		
18	¿Se ha desmayado alguna vez, quedando con problemas para caminar o ha tenido pérdida de fuerza en alguno de sus miembros?	☐
19	¿Ha perdido alguna vez la fuerza en alguno de sus brazos o piernas o en toda la mitad del cuerpo por tiempo prolongado?	☐
20	¿Ha presentado alguna vez “entumecimiento”, “adormecimiento” o pérdida de sensibilidad en la mitad de la cara o del cuerpo?	☐
21	¿Ha tenido dificultad para entender lo que dicen, para expresar lo que quiere decir o ha notado cambios en su voz?	☐
22	¿Ha tenido dificultad para tragar, visión doble o mareos, acompañado con dificultad para caminar, en forma transitoria o prolongada?	☐
23	¿Le han dicho alguna vez que ha tenido “derrame”?	☐
24	¿Ha visto borroso alguna vez o ha perdido bruscamente la visión en uno o ambos ojos?	☐
25	¿Le han dicho alguna vez que tuvo trombosis, hemorragia o “derrame”?	☐

Módulo: Enfermedad gingival		**Respuesta**	
26	¿Piensa usted que tal vez sufra de enfermedad de las encías?	1	Si
		2	No
		99	No sabe/no responde
27	En general, ¿cómo diría que es el estado de salud de sus dientes y encías?	1	Excelente
		2	Muy buena
		3	Buena
		4	Regular
		5	Mala
		99	No sabe/no responde
28	¿Alguna vez ha recibido tratamiento de las encías tipo raspado o alisado de las raíces, que a veces se conoce como “limpieza profunda”?	1	Si
		2	No
		99	No sabe/no responde
29	¿Alguna vez se le ha aflojado algún diente por si solo sin haber tenido una lesión?	1	Si
		2	No
		99	No sabe/no responde
30	¿Alguna vez un dentista le ha dicho que usted ha perdido hueso alrededor de los dientes?	1	Si
		2	No
		99	No sabe/no responde

Código del Participante:	☐☐☐ – ☐☐☐ – ☐☐	Código de trabajador:	☐☐

31	En los últimos tres meses, ¿ha notado usted un diente que no parece verse bien?	1	Si
		2	No
		99	No sabe/no responde
32	Aparte del cepillado de sus dientes, ¿cuántas veces ha usado hilo dental o algún otro medio o utensilio para limpiarse entre los dientes en los últimos siete días?	☐	Número de días
		99	No sabe/ no responde
33	Aparte del cepillado de sus dientes, ¿cuántas veces ha usado un enjuague bucal u otro producto líquido para el tratamiento de enfermedades o problemas dentales en los últimos siete días?	☐	Número de días
		99	No sabe/ no responde

Módulo: Síntomas autonómicos			
Síntoma/problema de salud		**Q1. Durante los últimos 6 meses, ¿ha tenido usted alguno de los siguientes síntomas?** 1 = Si → **Pasar a Q2** 2 = No	**Q2. ¿Cuánto diría usted que el síntoma le molesta?** 1 = No me molesta 2 = Un poco 3 = Algo 4 = Moderadamente 5 = Bastante
34	¿Tiene mareos?	☐	☐
35	¿Tiene la boca o los ojos secos?	☐	☐
36	¿Tiene sus pies pálidos?	☐	☐
37	¿Tiene los pies más fríos que el resto de su cuerpo?	☐	☐
38	¿Está el sudor de sus pies disminuido en comparación con el resto de su cuerpo?	☐	☐
39	¿Está el sudor de sus pies disminuidos o ausentes (por ejemplo, después de ejercicio o en clima cálido)?	☐	☐
40	¿Está el sudor en sus manos aumentado en comparación con el resto de su cuerpo?	☐	☐
41	¿Tiene nauseas, vómitos o distensión abdominal después de comer una comida pequeña?	☐	☐
42	¿Tiene diarrea persistente (más de 3 deposiciones blandas por día)?	☐	☐
43	¿Tiene estreñimiento persistente (más de 1 deposición cada dos días)?	☐	☐
44	¿Se le escapa la orina?	☐	☐
	Esta pregunta es solo para varones:		
45	¿Tiene dificultad para obtener una erección?	☐	☐

Código del Participante:	☐☐☐ – ☐☐☐ – ☐☐	Código de trabajador:	☐☐

Sección 7: Formato de Evaluación Cognitiva (CAF)

Módulo: Leganés		**Respuesta**		
Los problemas con la memoria preocupan mucho a los pacientes y a sus médicos. Disponemos de una prueba que consiste en una serie de preguntas que nos puede ayudar a diagnosticar esos problemas de memoria. Estas preguntas deberá responderlas usted solo, sin ayuda de su acompañante.				
Instrucciones: Por cada una de las siguientes preguntas, anotar la respuesta del participante, y colocar 1 en el puntaje si la respuesta es correcta y 0 si no es correcta.				
1	Por favor contésteme, ¿qué fecha es hoy?		**Respuesta**	**Puntaje**
		DD/MM/AÑO		
2	¿Qué hora es?		**Respuesta**	**Puntaje**
		Hora		
3	¿Qué día de la semana es?		**Respuesta**	**Puntaje**
		Día		
4	¿Cuál es su dirección completa?	**Respuesta**		**Puntaje**
5	¿En qué ciudad estamos?	**Respuesta**		**Puntaje**
6	¿Qué edad tiene?	**Respuesta**		**Puntaje**
7	¿Cuál es su fecha de nacimiento?	**Respuesta**		**Puntaje**
8	¿Cómo se llamaba su madre?	**Respuesta**		**Puntaje**
Instrucciones: Ahora le voy a enseñar algunos dibujos para que usted me diga lo que son:				
	Por cada una de las siguientes preguntas colocar 1 en el puntaje si la respuesta es correcta y 0 si no es correcta.	**Respuesta**		**Puntaje**
9	Vaca			
10	Barco			
11	Cuchara			
12	Avión			
13	Botella			
14	Camión			
Instrucciones: Por favor, repita que objetos ha visto e intente recordarlos porque dentro de un rato se los voy a volver a preguntar.				
	Dar un punto por respuesta correcta, si no dar cero	**Respuesta**		**Puntaje**
15	Vaca			
16	Barco			
17	Cuchara			
18	Avión			
19	Botella			
20	Camión			

Código del Participante:	☐☐☐ – ☐☐☐ – ☐☐	**Código de trabajador:**	☐☐

Instrucciones: Voy a leerle una historia corta. Preste mucha atención porque solo se la voy a leer una vez. Cuando haya terminado esperaré unos segundos y después le pediré que me diga todo lo que recuerda de ella. La historia es: (Leer despacio)

"Tres niños estaban solos en una casa y la casa se incendió. Un valiente bombero logró entrar por una ventana trasera y los llevó a un lugar seguro. Quitando pequeños cortes o rasguños todos estaban bien".

Instrucciones: Dar como máximo dos minutos para que diga lo que recuerda de la historia.

	Dar un punto por respuesta correcta, si no dar cero	**Respuesta**	**Puntaje**
21	Tres niños		
22	Casa se incendió		
23	Bombero entró		
24	Los niños fueron rescatados		
25	Pequeñas heridas		
26	Todos bien		

Instrucciones: Cinco minutos más tarde de que se le ensenaran los dibujos
(Durante este tiempo puede hacer una toma de presión arterial)

	¿Podría repetirme los objetos que vio en los dibujos hace un rato?	**Respuesta**	**Puntaje**
27	Vaca		
28	Barco		
29	Cuchara		
30	Avión		
31	Botella		
32	Camión		

Módulo: Tu memoria		**Respuesta**	
33	En comparación con hace 5 años, su memoria…	1	… ha mejorado
		2	… es la misma
		3	… es casi tan buena
		4	… está peor
		5	… está mucho peor

Código del Participante: ☐☐☐ – ☐☐☐ – ☐☐	**Código de trabajador:** ☐☐

Sección 8: Formato de Evaluación Antropométrica (AAF)

Fecha	
Fecha (DD-MMM-20AA)	☐☐ - ☐☐☐ - ☐☐

Talla		
Talla parado	☐☐☐.☐	[cm]

Peso				
Peso	☐☐☐.☐ [Kg]	Ropa:	☐	1 = Mínimo / No usa 2 = Ropa completa
Número de máquina	☐			

Circunferencias	**Medición 1**	**Medición 2**	**Medición 3**
Cintura (abdominal)	☐☐☐.☐ [cm]	☐☐☐.☐ [cm]	☐☐☐.☐ [cm]
Número del centímetro	☐		

Presión arterial [brazo]			
	Medición 1	**Medición 2**	**Medición 3**
Presión sistólica (brazo)	☐☐☐ [mm Hg]	☐☐☐ [mm Hg]	☐☐☐ [mm Hg]
Presión diastólica (brazo)	☐☐☐ [mm Hg]	☐☐☐ [mm Hg]	☐☐☐ [mm Hg]
Pulso	☐☐☐ [lat./min]	☐☐☐ [lat./min]	☐☐☐ [lat./min]
Manguito usado	☐ [1 = Pequeño; 2 = Mediano; 3 = Grande]		
Número de aparato	☐☐		
Medidas en lado derecho	☐ [1 = Si; 2 = No]		

Pupilómetro: Medidas				
		Escotópico	**L Mesópico**	**H Mesópico**
Ojo Derecho	Diámetro	☐☐.☐ [mm]	☐☐.☐ [mm]	☐☐.☐ [mm]
	STD	☐.☐☐ [mm]	☐.☐☐ [mm]	☐.☐☐ [mm]
Ojo Izquierdo	Diámetro	☐☐.☐ [mm]	☐☐.☐ [mm]	☐☐.☐ [mm]
	STD	☐.☐☐ [mm]	☐.☐☐ [mm]	☐.☐☐ [mm]

Código del Participante: ☐☐☐ – ☐☐☐ – ☐☐	**Código de trabajador:** ☐☐

Agudeza Visual		
	Ojo Derecho	**Ojo Izquierdo**
Angulo de resolución mínimo (MAR)	☐ . ☐ **LogMAR**	☐ . ☐ **LogMAR**

EZScan			
	Intolerancia a la glucosa: P[IGT]	**Resistencia a la insulina: P[IR]**	**Resultado final**
Porcentajes (%)	☐☐ **%**	☐☐ **%**	☐☐ **%**

Evaluación final	
Medidas adecuadas	☐ [1 = Si; 2 = No]
Si marcó NO, especificar	
Observaciones:	

APPENDIX G:

Table: Behavioural characteristics of the study population by sex

Behavioural characteristic		**Males** N = 754 **N (%)**	**Females** N= 750 **N (%)**
T2DM in first-degree relatives	Yes	226 (30.0%)	242 (32.3%)
Smoking			
Current smoking	Do not smoke	561 (74.4%)	734 (97.9%)
	Smoke occasionally	111 (14.7%)	12 (1.6%)
	Smoke daily	82 (10.9%)	4 (0.5%)
Self-reported history of smoking	Never smoked	241 (32.0%)	682 (90.9%)
	Smoked before	311 (41.2%)	54 (7.2%)
	Currently smoke	202 (26.8%)	14 (1.9%)
Alcohol use			
Alcohol consumption	Never	150 (19.9%)	468 (62.4%)
	< One per month	458 (60.7%)	278 (37.1%)
	1+ times per month	146 (19.4%)	4 (0.5%)
Alcohol disorder	Yes	119 (15.8%)	2 (0.3%)
Physical activity			
Physically active (≥ 30min/day)	Yes	557 (73.9%)	479 (63.9%)
MET score (IPAQ)	Low	179 (23.7%)	371 (49.5%)
	Moderate	240 (31.8%)	279 (37.2%)
	High	335 (44.4%)	100 (13.3%)
Watching television (hours/day)	< 2 hours/day	220 (29.2%)	321 (42.8%)
	≥2 but <4 hours/day	269 (35.7%)	244 (32.5%)
	4+ hours/day	265 (35.1%)	185 (24.7%)
Diet patterns			
Fruits and vegetables	At least one per day	356 (47.2%)	433 (57.7%)
Sweetened juices consumption	≥ Once per week	95 (12.6%)	62 (8.3%)
Soda consumption	≥ Once per week	166 (22.0%)	113 (15.1%)

APPENDIX H:

Table: Anthropometrical characteristics of the study population by sex

		Males	Females
		N = 754	N= 750
Anthropometric characteristic		**N (%)**	**N (%)**
Weight (kg)	Mean (SD)	75.8 (12.8)	69.1 (12.9)
Height (m)	Mean (SD)	1.67 (0.1)	1.54 (0.1)
Body mass index (kg/m^2)	Mean (SD)	27.1 (4.3)	28.9 (4.8)
Body mass index (categories)	Normal	245 (32.5%)	154 (20.5%)
	Overweight	340 (45.1%)	315 (42.0%)
	Obese	169 (22.4%)	281 (37.5%)
Waist circumference (cm)	Mean (SD)	93.8 (10.1)	93.4 (10.7)
Waist circumference (IDF categories)	Normal	253 (33.6%)	65 (8.7%)
	Obese	501 (66.4%)	685 (91.3%)
Systolic blood pressure (mmHg)	Mean (SD)	124.3 (14.9)	114.7 (16.3)
Diastolic blood pressure (mmHg)	Mean (SD)	81.0 (10.2)	77.9 (10.2)
Blood pressure treatment	Yes	41 (5.4%)	65 (8.7%)
Hypertension status	Yes	200 (26.5%)	170 (22.7%)

APPENDIX I:

Table: Comparison of results of risk scores and neuropathy assessment tools by sex

	Results by sex		
	Males (N = 754) Mean (SD)	**Females (N = 750)** Mean (SD)	**p-value***
Risk score			
FINDRISC	7.7 (4.1)	10.1 (3.9)	< 0.001
LA-FINDRISC	8.0 (4.3)	9.1 (4.5)	< 0.001
Peruvian Risk Score	1.5 (1.1)	1.4 (1.1)	0.06
Neuropathy assessment tool			
EZSCAN	25.7 (9.3)	28.8 (10.8)	< 0.001
Scotopic diameter	4.5 (0.9)	4.5 (0.8)	0.30
Low mesopic diameter	4.5 (0.8)	4.5 (0.8)	0.62
High mesopic diameter	4.3 (0.8)	4.3 (0.8)	0.29
Pulp of the hallux	16.5 (9.4)	14.2 (7.6)	< 0.001
First metatarsal head	14.5 (8.4)	12.9 (7.2)	< 0.001
Third metatarsal head	14.4 (8.7)	13.0 (7.4)	< 0.001
Fifth metatarsal head	14.3 (8.6)	12.8 (7.2)	< 0.001