GENERAL STUDY INFORMATION AND INFORMED CONSENT

UNIVERSITY CEU CARDENAL HERRERA

Principal investigator: Dr. Sergio Montero Navarro

TITLE: Carotid Wall Texture as a Cardiovascular Risk Biomarker in Type 2 Diabetes Mellitus

Research and Ethics Committee of CARDENAL HERRERA CEU UNIVERSITY: CEEI24/547

NCT ID:

DATE: 01-20-2026

GENERAL STUDY INFORMATION

TITLE: Carotid Wall Texture as a Cardiovascular Risk Biomarker in Type 2 Diabetes Mellitus

Mr. Sergio Montero Navarro, Physiotherapist, principal investigator and researcher reports that:

The tests performed are simple and in no case involve difficulty, fatigue, danger, injury, pain or adverse reaction.

They will be carried out by collegiate physiotherapists in the School of Physiotherapists of the Valencian Community.

The general data of the subject will be collected (name, age, sex, physical variables and clinical history). The article must be sent with comfortable clothes The day that sea cited by the researcher, previous notice. Personal data is recognized in this study.

The personal data are confidential, apply to the protection of personal data (Organic Law 15/1999, December 13) and any other thing that may be applicable.

This study was approved by the Research and Ethics Committee of CARDENAL HERRERA CEU UNIVERSITY: CEEI24/547

INFORMED CONSENT

Mr/Mrs .....with Number identification..... freely and voluntarily, I DECLARE:

That I have read the information contained in this document about the general information of the study.

I have been informed that all tests are simple to perform and do not produce harmful effects on health. They will be carried out in appropriate facilities and will be carried out by qualified and specialized personnel.

I have also been informed that, the data collected in this study will be treated confidentially, applying the current legislation on protection of personal data (Organic Law 15/1999, of December 13) and any other applicable.

Therefore, I give my consent and I authorize Mr. Sergio Montero Navarro, to carry out the detailed study in this document with the help of the necessary personnel with the appropriate qualification and specialization.

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SIGNED:

STUDY PROTOCOL PLAN AND STADISTICAL ANALYSIS PLAN (SAP)

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STUDY PROTOCOL PLAN

Objectives:

The objectives of this pilot study will be:

(1) to compare carotid intima–media thickness (CIMT) and layer-specific ultrasound texture characteristics of the common carotid artery between individuals with type 2 diabetes mellitus (T2DM) and normoglycemic controls;
(2) to evaluate the association between T2DM and these ultrasound-derived variables; and
(3) to assess the ability of CIMT and carotid wall texture features to discriminate between low and high 10-year cardiovascular risk, exploring their potential use as objective support tools for cardiovascular risk stratification and follow-up in preventive cardiovascular nursing, particularly in patients with T2DM.

Study Design

This study will be a double-blind, diagnostic accuracy study including two adult groups: participants with T2DM and healthy normoglycemic controls. The study will be conducted in accordance with the Standards for Reporting Diagnostic Accuracy Studies (STARD) and the principles of the Declaration of Helsinki.

Eligibility Criteria

Inclusion Criteria

- Adults aged 40 to 69 years
- European population
- No known cardiovascular disease
- Eligible for cardiovascular risk estimation using SCORE2 (control group) or SCORE2-Diabetes (T2DM group)

Exclusion Criteria

- Participants will be excluded if they present any of the following:
- History of cardiovascular disease (myocardial infarction, stroke, peripheral arterial

disease, or other diagnosed cardiovascular conditions)

- Advanced chronic kidney disease (estimated glomerular filtration rate <30 mL/min/1.73 m²)
- Chronic inflammatory or autoimmune diseases
- Chronic infections (e.g., HIV)
- Genetic or hereditary lipid disorders (e.g., familial hypercholesterolemia)
- Chronic liver disease (cirrhosis or significant hepatic steatosis)
- Chronic respiratory disease (e.g., COPD)
- Severe psychiatric or chronic neurological disorders
- Medical treatments that may substantially modify cardiovascular risk (e.g., systemic corticosteroids, chemotherapy)
- Morbid obesity (BMI >40 kg/m²)
- Extreme lifestyle factors (excessive alcohol intake, active substance abuse, or elite athletic training with atypical cardiovascular risk profiles)

Recruitment and Consent

Participants will be recruited from collaborating clinical laboratories following medical referral for routine blood testing. All participants will receive verbal and written information about the study and will provide written informed consent prior to enrollment. Recruitment and allocation will be conducted by a single investigator.

Blinding

Ultrasound acquisition will be performed by an investigator blinded to diabetes status.

Image processing and analysis will be conducted using anonymized data by an investigator blinded to participant identity, diabetes status, and cardiovascular risk.

Cardiovascular risk scores will be calculated independently by a third investigator blinded to ultrasound findings.

Ultrasound Assessment

Carotid ultrasound examinations will be performed using a LOGIQ-e R8 system (GE Healthcare, USA) with a linear 4–13 MHz transducer. Participants will be examined in the supine position after a standardized rest period.

Bilateral longitudinal images and video recordings of the common carotid artery will be acquired. End-diastolic frames will be extracted for CIMT measurement and texture analysis. All images will be stored in uncompressed TIFF format.

Image Analysis

Ultrasound images will be analyzed using ImageJ software. A standardized 1-cm region of interest (ROI) will be selected on the posterior wall of the common carotid artery proximal to the carotid bulb. Separate ROIs will be defined for the intima–media complex, media, adventitia, and total wall.

Texture analysis will be performed using first-order statistics and gray-level co-occurrence matrix (GLCM) features, including angular second moment, homogeneity, contrast, correlation, and entropy.

CIMT will be measured bilaterally at multiple locations within each ROI to improve precision and variability estimation.

Cardiovascular Risk Assessment

Ten-year cardiovascular risk will be estimated using SCORE2 (control group) and SCORE2-Diabetes (T2DM group) through the validated online calculator. Risk categories will be defined according to current European guidelines.

For analytical purposes, participants will be classified into two cardiovascular risk groups:

Low risk (low to moderate)

High risk (high to very high)

These classifications will serve as the clinical reference standard for evaluating the discriminative performance of ultrasound-derived variables.

Statistical Analysis Plan (SAP)

Data will be analyzed using IBM SPSS Statistics for Windows (v.25.0; IBM Company, 2017). JASP (v.0.18.3; JASP Team, 2024) will be used for graphical representation, and resampling analyses will be performed using R software (v.XXX; R Foundation for Statistical Computing, XXX). The normality and homoscedasticity of the variables will be assessed. When heteroscedasticity is detected, Welch's correction will be applied. Continuous variables will be summarized as mean ± standard deviation (SD) and 95% confidence intervals (CIs), while categorical variables will be reported as absolute and relative frequencies. Comparisons of continuous variables will be conducted using independent-samples t tests or the Mann–Whitney U test, as appropriate, and Fisher's exact test will be used for categorical variables. Effect size will be calculated using Cohen's d or rank-biserial correlation (r) in the case of non-parametric data. The Bonferroni correction will be applied when necessary. Statistical significance will be set at $\alpha = 0.05$.

The reference status will be defined according to the 10-year cardiovascular risk classification obtained using SCORE2 or SCORE2-Diabetes, categorizing participants into low- and high-risk groups. The diagnostic accuracy of carotid intima–media thickness (CIMT) and echotextural variables will be evaluated using logistic regression models adjusted for age, sex, and body mass index. Sensitivity (Se), specificity (Sp), the Youden index (Se + Sp − 1), and positive and negative likelihood ratios (LR+ and LR−) will be calculated. Discriminative ability will be assessed using receiver operating characteristics (ROC) curves. The area under the curve (AUC) will be estimated from the original sample, and its 95% confidence intervals (CIs) will be calculated using bootstrap resampling (2,000 iterations) to improve the stability of the estimates in the case of a pilot study. The 95% CIs for Se and Sp will be estimated using the exact binomial Clopper–Pearson method, which is appropriate for small sample sizes. An AUC value close to 0.90 and Se and Sp values ≥80% will be considered acceptable.