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Machine-Learning Prediction of Major Adverse Cardiovascular Events in Type 2 Diabetes With and Without Diabetic Foot Syndrome Using Surrogate Cardiovascular Risk Indices and Circulating Biomarkers: An Exploratory, Internally Validated Study

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MACHINE-LEARNING PREDICTION OF MAJOR ADVERSE CARDIOVASCULAR EVENTS IN TYPE 2 DIABETES WITH AND WITHOUT DIABETIC FOOT SYNDROME USING SURROGATE CARDIOVASCULAR RISK INDICES AND CIRCULATING BIOMARKERS: AN EXPLORATORY, INTERNALLY VALIDATED STUDY

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ABSTRACT

Background and aims. Diabetic Foot Syndrome (DFS) is a serious complication of diabetes, increasing mortality and cardiovascular risk. Although artificial intelligence (AI) is increasingly used in medicine, few studies have applied Machine Learning (ML) models combining clinical variables with cardiovascular, inflammatory, genetic and epigenetic markers in DFS. This study aimed to develop and internally validate ML models predicting major adverse cardiovascular events (MACE) in patients with type 2 diabetes with and without DFS, with hospitalisation as a secondary outcome, and to identify the variables most associated with MACE.

Methods. This retrospective analysis used the database of a single-centre observational cohort of 40 patients with type 2 diabetes and DFS and 40 age- and sex-matched diabetic controls without foot ulcers, enrolled in 2014–2015 and followed for at least 8 years. We analyzed clinical, cardiovascular (PWV, RHI, LF/HF), adipo-inflammation (Omentin-1, Gremlin-1, VEGF, HIF-1α), epigenetic (miR-217-5p, miR-503-5p) and genetic (VEGF −2578 C>A polymorphism) markers. Logistic Regression and Random Forest models were developed to predict MACE (non-fatal myocardial infarction, non-

fatal ischaemic stroke and all-cause death) and internally validated with stratified 5-fold cross-validation; no external validation was performed.

Results. DFS was associated with higher odds of MACE (OR 2.71, 95% CI 1.05–7.00; p=0.039). ML models showed moderate predictive performance (Random Forest AUC: 0.78, 95% CI 0.68–0.88), achieving maximal sensitivity through decision threshold recalibration. Key predictors identified included antiplatelet therapy, LF/HF ratio, VEGF levels, PWV, and endothelial function. In exploratory univariate analyses, patients with MACE had higher PWV, LF/HF and Gremlin-1 and lower VEGF ($p<0.05$).

Conclusions. In patients with type 2 diabetes, DFS was associated with higher odds of MACE over at least 8 years. An exploratory model combining conventional risk factors, vascular and autonomic indices (PWV, LF/HF) and circulating biomarkers (VEGF, Gremlin-1) showed moderate discrimination at internal validation, with no advantage of Random Forest over Logistic Regression.

These findings highlight the importance of comprehensive cardiovascular assessment and aggressive risk factor management in DFS patients, while supporting the potential role of ML in clinical decision-making. These hypothesis-generating findings require confirmation in larger cohorts with external validation.

Keywords: diabetic foot; type 2 diabetes mellitus; major adverse cardiovascular events; machine learning; random forest; arterial stiffness; endothelial dysfunction; heart rate variability; VEGF; Gremlin-1

1. BACKGROUND

Among diabetic vascular complications, foot ulcers are the major cause of hospitalization in patients with diabetes and a significant cause of health care costs (over 20%–40% of healthcare resources are reportedly allocated to diabetes-related foot care). Several studies indicate that diabetic foot ulceration is associated with substantial morbidity and mortality. A meta-analysis reported an approximately twofold increase in all-cause mortality among patients with diabetes and foot ulceration compared with patients with diabetes without foot ulceration [1]. Patients with diabetes and foot complications have been reported to have a worse cardiovascular and cerebrovascular risk profile, greater endothelial dysfunction and arterial stiffness, and a more pronounced inflammatory background than patients with diabetes without foot complications [2-4]. Diabetes-associated impairment of angiogenesis includes microRNA-mediated mechanisms [5], while DFS-specific studies have linked epigenetic alterations

with endothelial and adipo-inflammatory dysfunction [6]. In recent years, artificial intelligence has begun to spread its use in clinical practice.

However, despite the widespread use of artificial intelligence in the medical field, few studies in the literature have used this method in diabetic patients with chronic complications such as diabetes-related foot disease.

Indeed, Singh et al., in 2013, pioneered the use of AI in the context of diabetic foot ulcers. In that study, the role of five Toll-like receptor 4 (TLR4) polymorphisms in predicting the risk of diabetic ulcer occurrence was evaluated using an artificial neural network (ANN) [7].

Since 2020, there has been an increase in studies using artificial intelligence in diabetic patients with ulcerative complications of the lower limb to predict the diagnosis, prognosis, and treatment of this condition. Xie et al. devised a machine-learning model in 2022 to accurately estimate the rate of amputations in patients with diabetic ulcers [8].

In 2022, Du et al. used machine learning to assess mortality and amputation rates in patients with DFS during the COVID-19 pandemic and the post-pandemic era [9].

Khanna et al., in 2022, used deep learning to stratify cardio and cerebrovascular risk in patients with DFS [10]. To our knowledge, no machine learning analysis has examined the association of the occurrence of major adverse cardiovascular events (MACE: non fatal acute myocardial infarction, non fatal ischaemic stroke or all-cause death), and hospitalization rates with surrogate cardiovascular risk indices (PWV – pulse wave velocity, RHI – reactive hyperemia index, sympathetic vagal balance), with indices of cognitive decline (MMSE – mini mental state examination - and WMH – white matter hyperintensities - at brain MRI - magnetic resonance imaging), with serological markers of adipoinflammation (Omentin 1, Gremlin 1, VEGF – vascular endothelial growth factor, HIF-1α – hypoxia inducible factor 1) and with epigenetic modifications (miRNA 217-5p, miRNA 503-5p, VEGF C2578A).

Based on these premises, the primary aim of this study was to develop and internally validate machine learning models predicting MACE over a follow-up of at least 8 years, with hospitalisation as a secondary outcome, in a cohort of 40 diabetic patients with ulcerative complications of the lower limb, and in 40 age- and sex-matched diabetic controls without foot lesions. A secondary, exploratory objective was to examine the association of MACE with surrogate indices of cardio- and cerebrovascular risk (PWV, RHI, MMSE, WMH at brain MRI), serological markers of adipose inflammation (Omentin 1, Gremlin 1, VEGF, HIF-1α), and epigenetic (miR-217-5p, miR-503-5p) and genetic (VEGF −2578 C>A) markers.

2. METHODS

2.1 Study design and participants

The present study was a retrospective secondary analysis of the database generated by a single-centre observational cohort conducted at the Internal Medicine and Stroke Care Ward of the Policlinico "P. Giaccone" Hospital, University of Palermo. The machine-learning analyses were performed after completion of the observational data-collection period. The source database included participants enrolled between January 2014 and June 2015 (with a follow-up period extended until June 2023, ensuring a minimum follow-up duration of 8 years even for the last participants enrolled): 80 patients with type 2 diabetes mellitus, of which 40 patients hospitalised for diabetic ulcerative lesions of the lower limb and 40 diabetic patients without foot ulcers who were hospitalised for diabetes-related clinical conditions (e.g., glycaemic imbalance, hypoglycaemia, or skin lesions not involving the foot).
Of the 92 patients initially enrolled (45 with DFS and 47 without DFS), 12 (5 with DFS and 7 without DFS) were lost to follow-up, and no follow-up data were available for them (Figure 1). The remaining 80 patients (40 per group), with complete data, formed the analytical sample. Matching by age and sex was performed on the 92 enrolled patients. After the exclusion of the 12 patients lost to follow-up, the two analysed groups remained comparable for age and sex (Table 1).

Patients were eligible if they had type 2 diabetes mellitus and belonged to either the DFS group or the diabetic control group defined above. Patients with inflammatory or infectious diseases (except for infected ulcers), autoimmune or rheumatic diseases, neoplasms, haematological diseases, severe kidney or liver failure, fever, treatment with anti-inflammatory drugs, or hospitalisation within the preceding 3 months were excluded.

The ankle-brachial index (ABI) was measured at enrolment in all participants and patients with peripheral arterial disease, identified by a resting ABI ≤ 0.90, were excluded from both the DFS and control groups.

The DFS group comprised 40 patients with non-ischaemic diabetic foot ulcers associated with peripheral neuropathy. Ulcers were predominantly located on the plantar surface, particularly beneath the metatarsal heads, with additional lesions involving the toes and heel. Lesions varied in size and depth, ranging from superficial ulcers involving the skin to deeper lesions extending into the

subcutaneous tissue. Surrounding callus and hyperkeratosis were common, and pain was frequently reduced or absent owing to sensory impairment. Some ulcers showed clinical signs of local infection, including erythema, warmth, swelling, or purulent discharge. Ulcer characteristics were documented at enrolment, including anatomical location, area, depth, surrounding tissue changes, and infection status (according to the International Working Group on the Diabetic Foot - IWGDF) [11,12]. Diabetic peripheral neuropathy was assessed from the medical history, clinical examination, Neuropathy Symptom Score (NSS) [13], and Semmes-Weinstein monofilament testing for loss of protective sensation [14].

Type 2 diabetes mellitus was diagnosed according to the American Diabetes Association criteria: HbA1c ≥6.5%, fasting plasma glucose ≥126 mg/dL, 2-h plasma glucose ≥200 mg/dL during a 75-g oral glucose tolerance test, or random plasma glucose ≥200 mg/dL in a patient with classic symptoms of hyperglycaemia or hyperglycaemic crisis. Classification as type 2 diabetes also considered age at onset, clinical presentation, body weight, family history, timing of insulin initiation, and history of ketoacidosis [15]. Hypertension was defined according to the ESC/ESH criteria in force at enrolment [16]. Dyslipidaemia was defined by one or more of the following: triglycerides ≥150 mg/dL, low HDL-cholesterol (<40 mg/dL in men or <50 mg/dL in women), or LDL-cholesterol >116 mg/dL [17].
All participants provided written informed consent to participate and to permit use of their data. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Ethics committee approval and the protocol number are reported in the Declarations section.

2.2 Clinical, laboratory and molecular assessments

Clinical and anthropometric data were collected at enrolment. Participants were classified as having obesity (BMI ≥30 kg/m²), overweight (BMI 25.0–29.9 kg/m²), or normal weight (BMI 18.5–24.9 kg/m²). Blood samples collected at enrolment were used to measure alanine aminotransferase, triglycerides, blood glucose, total cholesterol, and HDL-cholesterol.
Serum concentrations of Gremlin-1 (RayBiotech), Omentin-1 (Biomatik), vascular endothelial growth factor (VEGF), and hypoxia-inducible factor 1α (HIF-1α; Thermo Fisher Scientific) were measured using the respective enzyme-linked immunosorbent assay kits.
The VEGF-2578 C>A single-nucleotide polymorphism (rs699947) was evaluated. DNA was extracted using a Qiagen kit and amplified with the StepOnePlus Real-Time PCR System; allelic variants were assigned from the corresponding fluorescence profiles. The miRNAs miR-217-5p, miR-503-5p, and

miR-126-3p were assessed after RNA purification and extraction, followed by quantitative real-time PCR using Qiagen reagents.

2.3 Cardiovascular and autonomic assessments

Carotid-femoral pulse wave velocity (PWV) was assessed in the supine position using a SphygmoCor system (version 7.1), in accordance with established methodological recommendations. PWV was calculated as the distance travelled by the arterial pulse wave divided by the transit time between the carotid and femoral recording sites and was expressed in metres per second [18,19].

Peripheral endothelial function was assessed by reactive hyperaemia peripheral arterial tonometry (RH-PAT) using the EndoPAT 2000 system (software version 3.0.4). The reactive hyperaemia index (RHI) was calculated automatically as the post-occlusion-to-baseline pulse-amplitude ratio in the test arm, normalised to the corresponding ratio in the control arm and corrected for baseline vascular tone. An RHI value <1.67 was considered indicative of endothelial dysfunction [20,21].

Twenty-four-hour ECG Holter monitoring was performed using Sorin SpiderView digital recorders and analysed with ELA Medical SyneScope software (version 3.10). Time-domain heart-rate variability (HRV) measures included average heart rate, RMSSD, SDNN, and SDANN/5 min; frequency-domain measures included HF%, LF%, and the LF/HF ratio.

2.4 Cognitive and brain MRI assessments

The Mini-Mental State Examination (MMSE), an 11-question instrument, was administered to assess language, attention, orientation, memory, repetition, and calculation. The maximum score was 30, and values <24 were considered suggestive of cognitive impairment [22].

Brain MRI was used to assess white-matter abnormalities. Periventricular hyperintensities and deep white-matter hyperintensities were graded from 0 to 3 using the Fazekas score [23].

2.5 Outcomes, follow-up and statistical/machine-learning analysis

Outcomes and ascertainment. MACE were defined as a composite of non-fatal acute myocardial infarction, non-fatal ischemic stroke, or death from any cause (a modified three-point MACE, with all-cause instead of cardiovascular death). Hospitalisation was defined as an emergency or planned hospital admission for metabolic decompensation, cardiovascular conditions, or complications of diabetic foot lesions.

Events were ascertained through telephone interviews with patients or, for deceased patients, their relatives. Events were verified from discharge letters, death certificates or registries.

Follow-up. The source database covered the period between January 2014 and June 2015, with a follow-up period extended until June 2023, ensuring a minimum follow-up duration of 8 years even for the last participants enrolled.

Twelve participants (5 with DFS and 7 without DFS) were lost to follow-up with no follow-up data available and were excluded from the analysis (Figure 1).

The final analytical dataset comprised 80 patient records. MACE and hospitalisation were treated as separate binary outcomes. No missing values were identified, participants with incomplete data were excluded. No formal sample-size calculation was performed; with 29 events and 44 candidate predictors the analysis is exploratory [24]. Numerical variables were standardised using StandardScaler to prevent variables with larger magnitudes from disproportionately influencing the models.

Conventional statistical analysis. The unadjusted difference in MACE occurrence between groups was assessed using the chi-squared test, and logistic regression was used to estimate the association between DFS and MACE, reported as odds ratios with 95% CIs, without adjustment for covariates. Continuous variables were compared with the Mann–Whitney U test and categorical variables with the chi-squared or Fisher's exact test.

Machine-learning analysis compared Logistic Regression with Random Forest. To address the moderate class imbalance (29 events vs 51 non-events) without data leakage, the Synthetic Minority Over-sampling Technique (SMOTE) was applied only within the training folds of stratified 5-fold cross-validation; performance was evaluated on the original, non-synthetic validation folds. The primary discrimination metric was the area under the receiver operating characteristic curve (ROC AUC). The 95% confidence interval (CI) of the AUC was estimated with the DeLong method.

Random Forest hyperparameters, including n_estimators, max_depth, min_samples_split, and class_weight, were optimised using GridSearchCV with stratified 5-fold cross-validation and ROC AUC scoring. The grid of candidate values and the selected values are reported in Figure 1. Tuning and performance estimation used the same cross-validation scheme, without nested cross-validation. After hyperparameter tuning, the model was refitted and Gini importance was extracted for each variable. Features were ranked by importance, and the 15 highest-ranking features for MACE were selected for subsequent exploratory univariate comparisons.

For the final optimised model, the default decision threshold of 0.5 was recalibrated using out-of-fold probability estimates. A precision-recall curve was constructed, and the threshold maximising the F1-score was selected. Performance at this threshold was summarised using a confusion matrix and a classification report including sensitivity (recall), specificity, precision and negative predictive value. Because the threshold was selected on the same out-of-fold predictions used to evaluate it, these estimates are optimistic. The study is reported in accordance with the TRIPOD+AI [25] and STROBE [26] statements.

3. RESULTS

Baseline characteristics are reported in Table 1. MACE occurred in 29 of 80 patients (36%) 19/40 (47.5%) in DFS vs 10/40 (25%) in controls; 8 non-fatal myocardial infarctions, 4 non-fatal ischaemic strokes and 7 deaths in DFS vs 5, 2 and 3 in controls. The difference in MACE incidence between groups was not statistically significant with the chi-squared test (p=0.0628, Yates-corrected), whereas in univariable logistic regression DFS was associated with higher odds of MACE (OR 2.71, 95% CI 1.05–7.00; p=0.039) (Figure 2).

In the comparative machine learning phase, both Logistic Regression and Random Forest models demonstrated moderate and virtually identical predictive performance. The 5-fold cross-validation yielded a mean ROC AUC of 0.777±0.084 for Logistic Regression and 0.776±0.089 for Random Forest. ROC curves generated from the cross-validated predictions confirmed this similarity, with an integrated AUC of 0.76 (95% CI 0.65–0.88) for Logistic Regression and 0.78 (95% CI 0.68–0.88) for Random Forest. (Figure 3).

Hyperparameter tuning of the Random Forest model did not improve discrimination (best cross-validation ROC AUC 0.776). The final ROC curve generated from the optimized model's cross-validated predictions yielded an AUC of 0.78 (95% CI 0.68–0.88). The decision threshold was then adjusted.

By maximizing the F1-score, a threshold of 0.35 was selected (F1-score 0.725). At this threshold, sensitivity for MACE was 1.00. This configuration successfully identified all 29 MACE-positive cases (true positives 29; false negatives 0), with 22 false positives and 29 true negatives (specificity 0.57, negative predictive value 1.00); overall, 51 of 80 patients (64%) were classified as high risk. Precision

was 0.57. Because the threshold was selected on the same out-of-fold predictions, these estimates are optimistic. (Figure 4).

The five features with the highest Gini importance were antiplatelet agents, LF/HF, VEGF, pulse wave velocity, and endothelial function (RHI <1.67). Exploratory univariate analyses were subsequently performed to examine differences in these and other candidate variables according to MACE status. The use of antiplatelet agents (OR 8.39 ; p=0.0001) and dyslipidaemia (OR 3.90; p=0.0117) were associated with higher odds of MACE. Patients who experienced MACE had higher pulse wave velocity (p=0.0091), LF/HF (p=0.0384), Gremlin-1 (p=0.0332), weight (p=0.0387) and mean diastolic blood pressure (p=0.0437). VEGF levels were lower in patients with MACE (mean 54.51 vs 77.53 pg/mL, p=0.0109). These p-values are not adjusted for multiple comparisons, and several would not remain significant after correction. (Figure 5).

Mortality (7/40 in DFS vs 3/40 in controls; p=0,518) and hospitalisation (21/40 vs 17/40; p=0,502) did not differ significantly between the two groups. MMSE scores, white-matter hyperintensities, omentin-1, HIF-1α, miR-217-5p, miR-503-5p and the VEGF −2578 C>A genotype according to MACE status are reported in Table 1.

4. DISCUSSION

In this cohort of patients with type 2 diabetes followed for at least 8 years, DFS was associated with higher odds of MACE (OR 2.71, 95% CI 1.05–7.00), although the crude between-group difference did not reach statistical significance with the Yates-corrected chi-squared test (p=0.0628) and the confidence interval was wide.

Previous research on this cohort has highlighted that patients with DFS exhibit a higher degree of surrogate cardiovascular risk than diabetic patients without mention of ulcerative complications of the lower limbs [4]. In fact, these patients had a higher degree of arterial stiffness, assessed by PWV, and a higher degree of endothelial dysfunction, assessed by RHI analysis. Furthermore, DFS had a higher background of adipose inflammation as assessed by serum levels of inflammatory adipokines such as omentin 1 and gremlin 1 [6,27]. Moreover, additional studies have shown that patients with DFS, compared to patients with diabetes, exhibit a greater number and severity of white matter lesions on brain MRI. These patients, therefore, had greater cerebrovascular damage, both instrumental and clinical, as demonstrated by the greater degree of mild cognitive impairment, and the presence of DFS

was an additional factor of vascular damage in a clinical context of cerebral microvascular damage [23].

The higher cardiovascular vulnerability associated with DFS may partly reflect the coexistence of endothelial dysfunction, arterial stiffness, microvascular damage, and adipose inflammation. Although the present analysis cannot establish a causal relationship, these abnormalities may contribute to the adverse cardiovascular profile observed in patients with DFS. Collectively, these findings support the potential value of intensified cardiovascular risk assessment and optimization of modifiable risk factors in patients with DFS.

Current research exhibits a significant gap concerning ML-based MACE prediction within the DFS population. By addressing this oversight—especially regarding comparisons with diabetic controls—the present study provides novel, exploratory data. The optimized Random Forest model identified Antiplatelet agents, the LF/HF ratio, Vascular Endothelial Growth Factor (VEGF), pulse wave velocity (PWV), and endothelial function as the five most influential features associated with MACE.

Previous findings from our group showed that patients with DFS had higher PWV and lower RHI values than diabetic patients without ulcerative complications, indicating greater arterial stiffness and endothelial dysfunction. Alterations in the LF/HF ratio were also associated with DFS and correlated with endothelial function, supporting a potential interaction between cardiovascular autonomic regulation and vascular dysfunction [27]. In the present cohort, patients who experienced MACE showed higher LF/HF and PWV values, while measures of endothelial function were among the most influential features in the Random Forest model.

Although LF/HF was previously found to be lower in patients with DFS than in diabetic controls, the present analysis showed higher LF/HF values among patients who experienced MACE. These apparently divergent findings derive from different clinical comparisons and may indicate that cardiovascular autonomic dysfunction is not adequately represented by a simple linear increase or decrease in the LF/HF ratio. Chance (the LF/HF difference was not corrected for multiple comparisons) and confounding by treatment, arrhythmias or cardiac autonomic neuropathy may also contribute.

Overall, these findings suggest that arterial stiffness, endothelial dysfunction, and altered autonomic balance may represent complementary components of the cardiovascular risk phenotype associated with DFS and MACE. However, their individual prognostic contribution requires confirmation in larger and externally validated cohorts.

Regarding VEGF, a previous study demonstrated higher serum VEGF levels in patients with DFS than in patients with diabetes without ulcerative complications [6]. VEGF is a proangiogenic mediator

involved in diabetic-ulcer healing and re-epithelialization, and VEGF signalling promotes neoangiogenesis and endothelial nitric oxide synthase-mediated vasodilation [28-30]. Genetic variants in VEGF have also been associated with susceptibility to diabetic foot ulceration [31]. Increased VEGF concentrations have been reported after acute myocardial infarction [30,32]. The lower VEGF levels in patients with MACE contrast with the higher levels previously observed in DFS [6], although DFS was itself associated with MACE; this apparent paradox may reflect group composition and the influence of active ulcer healing on circulating VEGF at enrolment, and should be explored with analyses stratified by, or adjusted for, DFS status.

However, the present finding of lower systemic VEGF levels among patients who experienced MACE may reflect an impaired compensatory angiogenic response in advanced diabetic vascular disease. Diabetes-associated impairment of angiogenic signalling and reduced cardiac expression of VEGF and its receptors may contribute to impaired collateral formation; nevertheless, this mechanistic interpretation remains hypothetical and cannot be demonstrated by the present observational analysis [5,30,33].

The combination of its high feature importance in the Random Forest model and the significantly lower concentrations observed in MACE-positive patients identifies VEGF as a potentially relevant component of the cardiovascular risk profile. Nevertheless, this finding remains hypothesis-generating and requires external confirmation.

The statistically significant association between antiplatelet therapy and MACE requires cautious interpretation. Antiplatelet and antithrombotic therapies reduce cardiovascular events in appropriately selected patients with diabetes and symptomatic peripheral artery disease, but substantial residual risk may persist [34-36]. Patients with diabetic foot disease, particularly those with deep or infected ulcers, also have a high baseline risk of mortality and cardiovascular events [37,38]. Therefore, the observed association is likely to be influenced by confounding by indication, because patients with established or more severe cardiovascular disease are both more likely to receive antiplatelet therapy and more likely to experience subsequent MACE. Antiplatelet therapy is therefore best regarded as a marker of established cardiovascular disease, which should be considered as a candidate predictor or adjustment variable in future models. Diabetes-related thrombo-inflammation and increased platelet reactivity may further contribute to residual cardiovascular risk [39]. Dyslipidaemia, particularly elevated LDL cholesterol, is an established contributor to atherosclerotic cardiovascular risk in diabetes and was associated with MACE in the present cohort [17].

Patients who experienced MACE also showed higher weight, diastolic blood pressure, and Gremlin-1 concentrations. Although weight and blood pressure are established cardiovascular risk factors, the present observational findings demonstrate associations rather than causal effects.

Gremlin-1 is an inflammatory adipokine produced by adipose tissue that antagonises insulin action and is increased in type 2 diabetes and NAFLD/NASH [40]. Experimental studies indicate that Gremlin-1 can modulate VEGFR2 signalling in a context-dependent manner [41,42], whereas higher plasma Gremlin-1 levels have been associated clinically with an increased risk of acute coronary syndrome in patients with type 2 diabetes [43]. In a previous study, patients with DFS had elevated serum Gremlin-1 levels [6]. These findings support Gremlin-1 as a marker of adipose inflammation and a potential marker of cardiovascular risk, but they do not establish a causal role in MACE.

From a clinical perspective, the present findings suggest that cardiovascular risk stratification in patients with DFS may benefit from the integration of conventional risk factors with measures of vascular function, autonomic balance, and circulating biomarkers. Nevertheless, the proposed profile should be considered exploratory until it is confirmed through external validation.

For the detection of potentially fatal outcomes such as MACE, a high-sensitivity threshold may be clinically advantageous because it minimizes false-negative classifications. In the present cohort, the optimized threshold identified all MACE-positive patients, although this benefit was achieved at the expense of lower precision and a greater number of false-positive classifications. Therefore, its clinical utility requires prospective and external validation.

The present study has several strengths. First, the prolonged follow-up period of at least eight years enabled the assessment of long-term cardiovascular outcomes in a clinically well-characterized population of patients with type 2 diabetes, with and without DFS.

Second, the analysis integrated conventional cardiovascular risk factors with vascular, autonomic, and circulating biomarker measurements, thereby capturing multiple complementary dimensions of cardiovascular risk.

Third, the direct comparison between Logistic Regression and Random Forest allowed the performance of a conventional interpretable model to be evaluated against that of a nonlinear machine learning approach. The use of cross-validated predictions avoided reporting training-set performance, although hyperparameter tuning and threshold selection on the same folds may still yield optimistic estimates. Notably, the similar discrimination achieved by the two models suggests that increased algorithmic

complexity did not provide a substantial predictive advantage in this cohort, an observation that may favor the use of more parsimonious and interpretable approaches.

Finally, the identification of lower VEGF levels and higher Gremlin-1 concentrations among patients who experienced MACE provides hypothesis-generating evidence regarding the possible contribution of impaired angiogenic compensation and adipose inflammation to residual cardiovascular risk in DFS.

5. LIMITATIONS

The present study has several limitations that should be acknowledged. First, the sample size (n=80) appears relatively limited for a Machine Learning analysis involving a high number of clinical and biochemical variables. With 29 events and 44 candidate predictors, the number of events per candidate predictor was far below recommended minima [24], so the model is at high risk of overfitting; cross-validation does not remove this risk, and SMOTE, applied to address class imbalance, may miscalibrate predicted probabilities [44]. The predictive model therefore requires validation in larger, multi-center cohorts to confirm external validity. Second, the study follows a monocentric design, involving patients from a single University Hospital, which may limit the generalizability of the findings to other populations with different genetic or environmental backgrounds.

Furthermore, while 5-fold cross-validation was employed to stabilize performance estimates, the high sensitivity observed may vary in larger, more diverse external cohorts. Third, events were ascertained by telephone interview and, although verified against discharge letters, death certificates or registries, some misclassification cannot be excluded. Fourth, outcomes were analysed as binary variables, without accounting for time to event, competing risks or the matched design. Fifth, the association between DFS and MACE was not adjusted for potential confounders, and the association between antiplatelet therapy and MACE is likely confounded by indication. Sixth, model calibration was not assessed, and hyperparameter tuning and threshold selection were performed on the same cross-validation folds used to estimate performance, although tuning did not improve the cross-validated AUC of the Random Forest (0.776 before and after tuning). Seventh, biomarkers and vascular indices were measured once, at enrolment during hospitalisation, and treatment changes over follow-up were not captured. Eighth, 12 of the 92 enrolled patients (13%) were lost to follow-up and excluded, which may have introduced selection bias. Ninth, although patients with an ABI $\leq$0.90 were excluded, ABI alone cannot reliably rule out peripheral arterial disease in patients with diabetes, so residual confounding by undetected peripheral arterial disease remains possible. Finally, multiple exploratory comparisons were not corrected for multiplicity.

6. CONCLUSIONS

In conclusion, Diabetic Foot Syndrome was associated with higher odds of Major Adverse Cardiovascular Events over at least 8 years in patients with type 2 diabetes. In an exploratory machine learning analysis with moderate discrimination at internal validation, the features most associated with MACE included, besides traditional risk factors and antiplatelet therapy, the LF/HF ratio, arterial stiffness (PWV), and the circulating biomarkers VEGF and Gremlin-1.

To date, there are few studies in the literature that have presented machine learning models capable of predicting MACE in patients with diabetes. Furthermore, to our knowledge, machine learning models for predicting MACE in patients with DFS, compared with diabetic controls, have not previously been reported.

These results are consistent with the hypothesis that autonomic dysfunction, endothelial damage and chronic inflammation contribute to the elevated cardiovascular risk of patients with foot ulceration, but they do not establish causality. A high-sensitivity threshold could make such a model useful for screening; in this cohort, however, it classified about two thirds of patients (51 of 80) as high risk, and its clinical utility requires prospective and external validation.

Our results emphasise the clinical concept that the elevated cardiovascular risk in patients with foot ulceration is driven by a complex interplay of autonomic dysfunction, endothelial damage, and chronic inflammation. but they do not establish causality. A high-sensitivity threshold could make such a model useful for screening; in this cohort, however, it classified about two thirds of patients (51 of 80) as high risk, and its clinical utility requires prospective and external validation.

Consequently, the implementation of rigorous cardiovascular screening protocols and the aggressive management of modifiable risk factors appear warranted in this vulnerable population to improve long-term prognosis.

Machine learning may help integrate multifaceted clinical and biological data for cardiovascular risk stratification in DFS; in this cohort, however, it offered no advantage over logistic regression, and validation in larger, multicentre cohorts is required before these predictors can inform clinical practice.

Consent for publication: All enrolled patients (or legal parents or guardians for children) provided consent to publish their individual patient data.

Availability of data and materials: the datasets analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: no potential conflicts of interest relevant to this article were reported.

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Ethics declaration: This protocol study was approved by the Ethics Committee of the Policlinico P. Giaccone Hospital, and all patients provided written informed consent to participate in the study. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and written informed consent was obtained from all patients. Ethics approval was obtained using the name of the ethics committee and reference number if appropriate.

Authors' contributions: S.F. and A.D.C. designed the study, coordinated the research group, and contributed to manuscript preparation and revisions; S.F. statistical analysis; G.P., M.M.M., M.G.P., E.P., V.D.C., R.R., M.G.B., C.D.M., T.D.C., R.M.P., L.A., and D.D.R. collaborated to collect and process data; M.C., S.G. and A.T. collaborated on supervision and manuscript revisions.

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FIGURES

Figure 1. Study population

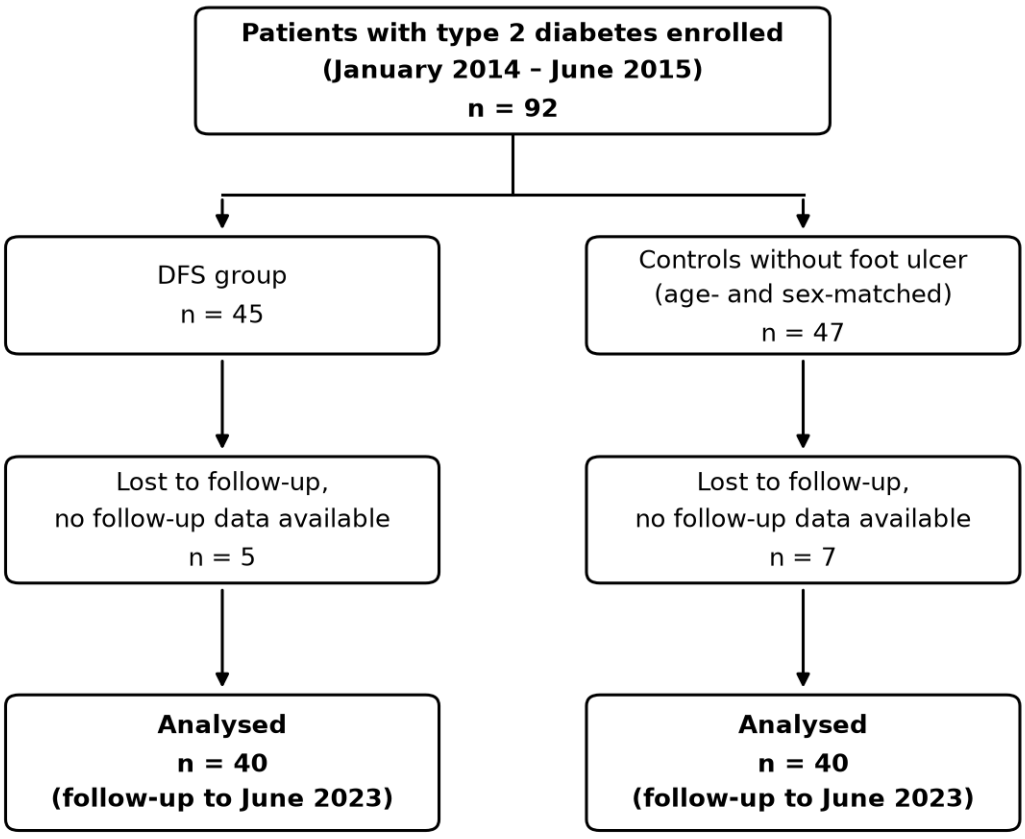


Table 1. Baseline characteristics of the study population

Comparison between the diabetic foot syndrome (DFS) group and the type 2 diabetes mellitus (DM2) group.

Variable	DFS (n=40)	DM2 (n=40)	P value
Continuous variables			
Systolic BP (mmHg)	133.12 ± 19.80	124.50 ± 17.86	**0.044**
Diastolic BP (mmHg)	68.20 ± 10.36	72.50 ± 12.96	0.091
Height (cm)	170.82 ± 8.94	169.18 ± 9.23	0.419
Weight (kg)	89.78 ± 15.47	85.50 ± 20.95	0.220
BMI (kg/m²)	30.75 ± 4.66	29.69 ± 6.30	0.242
PWV (m/s)	9.74 ± 3.55	8.46 ± 3.32	0.060
RHI	1.84 ± 1.47	1.79 ± 0.58	0.194
AIX	20.70 ± 15.79	12.97 ± 12.76	**0.019**
MMSE	26.52 ± 3.17	27.52 ± 2.30	0.231
HbA1c (%)	8.2 ± 1.3	7.3 ± 1.4	**<0.001**
PVH	1.27 ± 0.78	0.68 ± 0.66	**<0.001**
DWMH	0.80 ± 0.85	0.33 ± 0.66	**0.003**
Fazekas score	0.78 ± 0.73	0.62 ± 0.63	0.408
RMSSD (ms)	85.82 ± 96.91	38.52 ± 36.22	**0.042**
SDANN/5 min (ms)	63.22 ± 31.06	58.12 ± 15.13	0.444
SD (ms)	87.33 ± 58.12	78.33 ± 28.81	0.821
LF (%)	18.00 ± 10.81	23.08 ± 12.45	**0.013**
HF (%)	23.12 ± 15.73	10.34 ± 10.65	**<0.001**
LF/HF	1.19 ± 0.81	3.20 ± 2.86	**<0.001**
Omentin-1 (ng/mL)	19.65 ± 3.86	20.98 ± 6.09	0.424
GREM-1 (ng/mL)	1.74 ± 0.51	1.28 ± 0.21	**<0.001**
HIF-1α (pg/mL)	39.89 ± 10.79	34.81 ± 7.01	**0.003**
VEGF (pg/mL)	194.49 ± 202.29	98.47 ± 44.85	**0.015**
Hsa-miR-217-5p	13.58 ± 0.28	13.50 ± 0.00	**0.006**
Hsa-miR-503-5p	11.61 ± 2.16	13.22 ± 0.63	0.662
Dichotomous variables, n (%)			
Sex	27 (67.5)	22 (55.0)	0.251
Endothelial function	25 (62.5)	19 (47.5)	0.178
Hypertension	38 (95.0)	28 (70.0)	**0.003**
IMA	8 (20.0)	5 (12.5)	0.546
Stroke	4 (10.0)	2 (5.0)	0.675
Dyslipidemia	30 (75.0)	17 (42.5)	**0.003**
Smoking	16 (40.0)	13 (32.5)	0.485
Beta-blockers	17 (42.5)	18 (45.0)	0.822
Calcium-channel blockers	11 (27.5)	11 (27.5)	1.000
ACE-I/ARBs	21 (52.5)	19 (47.5)	0.655
Statins	26 (65.0)	20 (50.0)	0.175
Antiplatelet therapy	25 (62.5)	13 (32.5)	**0.007**
GLP-1/DPP-4 inhibitors	5 (12.5)	5 (12.5)	1.000
Sulfonylureas	2 (5.0)	5 (12.5)	0.432
Metformin	6 (15.0)	20 (50.0)	**<0.001**
Insulin	31 (77.5)	10 (25.0)	**<0.001**
MACE	19 (47.5)	10 (25.0)	**0.036**
Hospitalization	21 (52.5)	17 (42.5)	0.502
Morte	7 (17.5)	4 (10.0)	0.518
VEGF C2578A CC	26 (65.0)	28 (70.0)	0.633
VEGF C2578A AC	5 (12.5)	5 (12.5)	1.000
VEGF C2578A AA	9 (22.5)	7 (17.5)	0.576

Figure 2. Logistic Regression: Impact of DFS on MACE

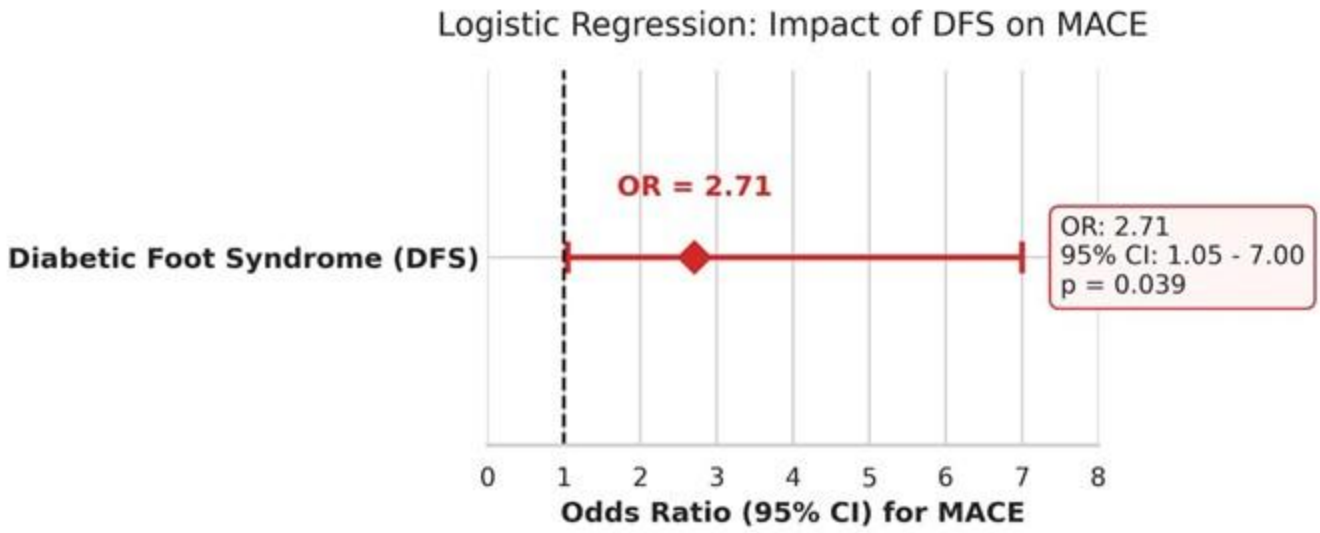


Figure 3. Cross Validation Analysis of Logistic Regression Model and Random Forest Model

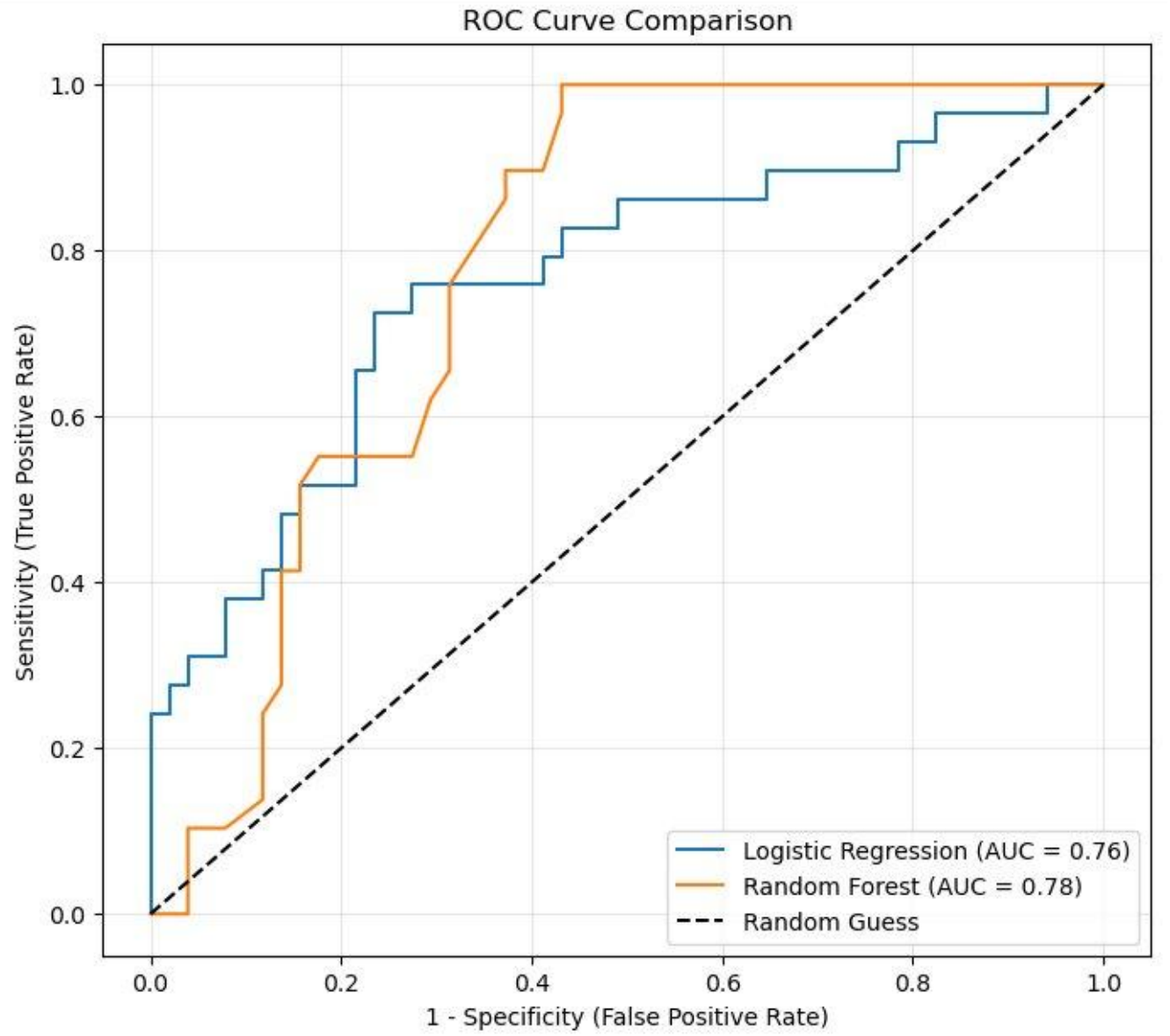


Figure 4. Confusion matrix analysis.

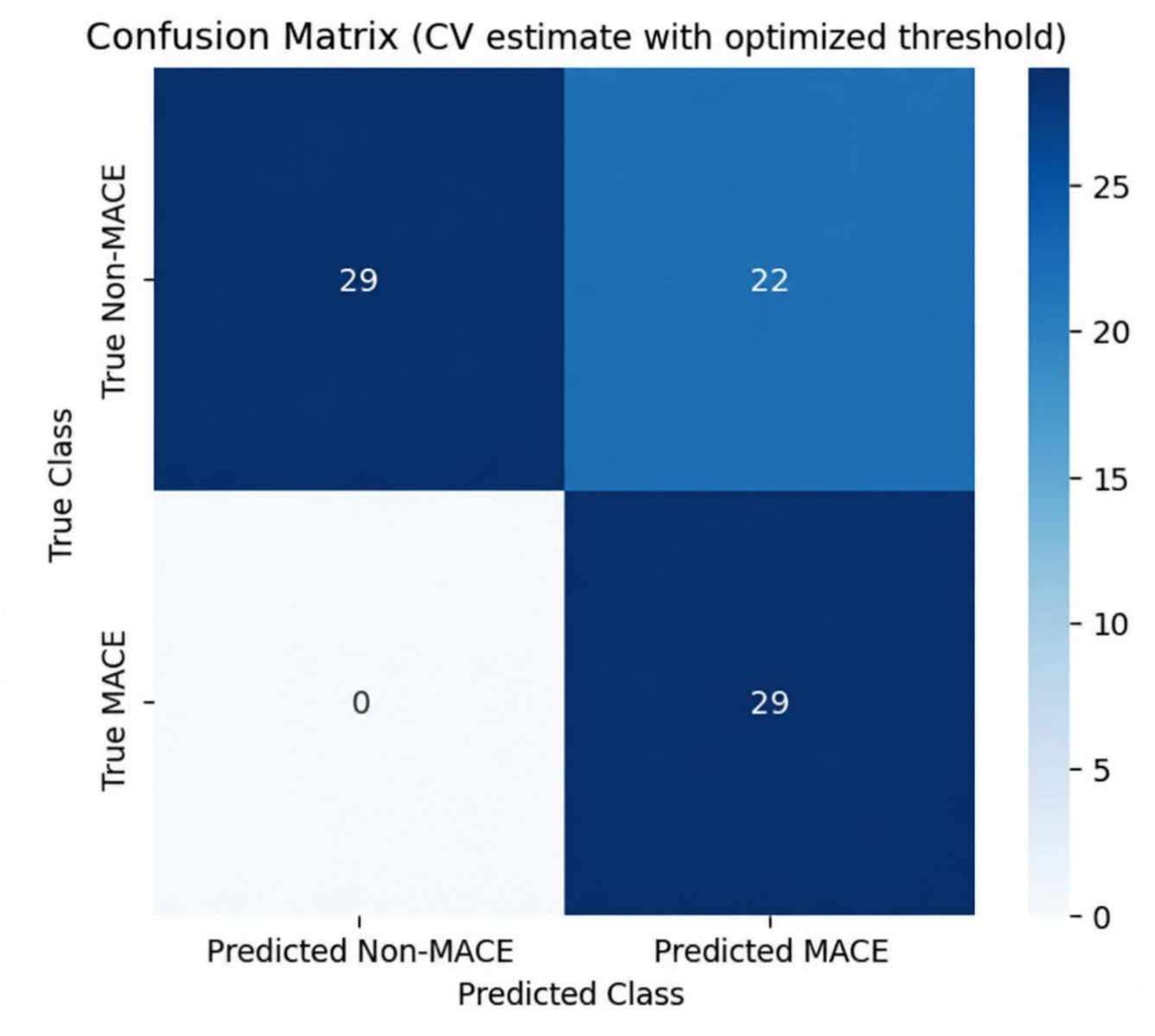
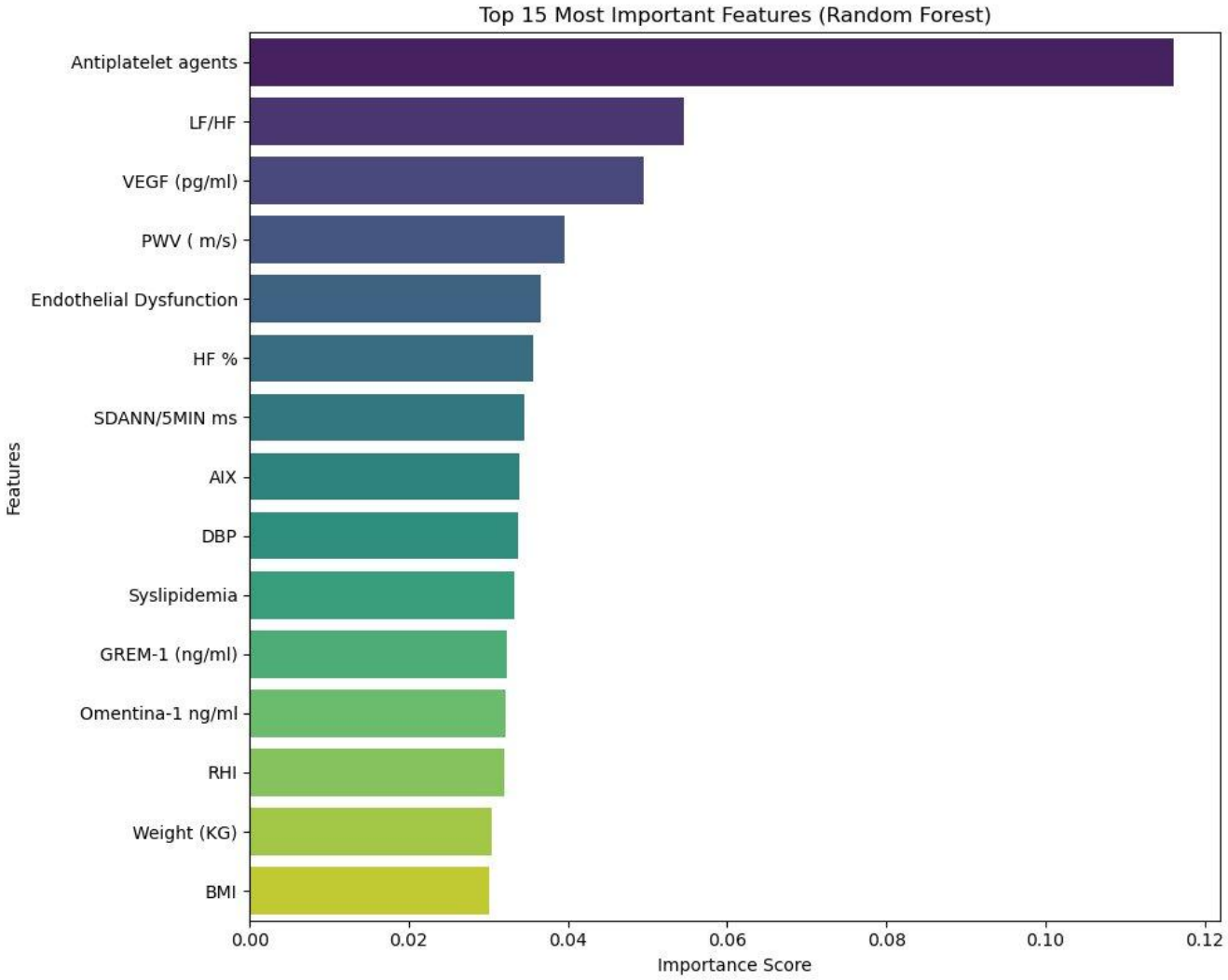


Figure 5. Most important Features with Random Forest


Top 15 Most Important Features (Random Forest)
Antiplatelet agents
LF/HF
VEGF (pg/ml)
PWV (m/s)
Endothelial Dysfunction
HF %
SDANN/5MIN ms
AIX
DBP
Syslipidemia
GREM-1 (ng/ml)
Omentina-1 ng/ml
RHI
Weight (KG)
BMI
Features
0.00
0.02
0.04
0.06
0.08
0.10
0.12
Importance Score