Special Issue Reprint

# Diabetes, Hypertension, and Cardiovascular Disease

Clinical Insights, Mechanisms, and Pharmacotherapies

Edited by
Ming-Jui Hung

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Editor

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# About the Editor

**Ming-Jui Hung**

Dr. Hung received his M.D. degree from Chung Shan Medical University, Taichung in 1990 and his Ph.D. degree from the Graduate Institute of Clinical Medical Sciences at the Chang Gung University College of Medicine, Taoyuan in 2010. Between his M.D. and Ph.D. degrees, he completed his residency training in internal medicine at Chang Gung Memorial Hospital in Linkou, Taoyuan and his fellowship training in cardiology at Chang Gung Memorial Hospital in Keelung. He was appointed General Attending Physician and Assistant Professor in 2001 at Chang Gung Memorial Hospital in Keelung and promoted to Professor in 2013. Dr. Hung's main research and clinical interests are coronary vasospasm and echocardiography. He has published more than 180 papers in prestigious journals with high scientific impact. Dr. Hung's research interests are focused on eNOS regulations that govern vascular function, especially in dynamic coronary stenosis. His laboratory attempts to apply basic findings about the vascular wall to the possible pathophysiologic mechanism in dynamic coronary stenosis. His laboratory studied the interaction between eNOS and caveolin-1 during inflammation and found that chronic inflammation transiently attenuates the expression of eNOS and permanently accentuates caveolin-1 after IL-6 administration in human umbilical vein cells. His catheterization laboratory is the first to study ROCK activity in patients with coronary spasm in Taiwan. Dr. Hung is also interested in coronary flow reserve using echocardiography. His echocardiographic laboratory examines the coronary flow reserve and left ventricular anatomical and functional changes in patients with chronic kidney disease. Dr. Hung is currently studying left ventricular and left atrial changes using echocardiography in a longitudinal study on patients with chronic kidney disease. His research efforts are currently supported by grants from several hospitals and Taiwan's national science council.

*Editorial*

# Diabetes, Hypertension and Cardiovascular Disease: Clinical Insights, Mechanisms and Pharmacotherapies

**Ming-Jui Hung** [1,2]

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Cardiovascular disease (CVD) is a serious issue demanding world attention, not only because of its role in increased mortality, but also in conjunction with the aging population and growing prevalence of other co-morbidities, such as hypertension, diabetes, etc. In 2019, CVD was responsible for 33% of all-cause deaths [1]. For many years, social and academic groups have vigorously emphasized the importance of lifestyle and behavior adjustment [2]. Approximately 19.1% and 22.2% of deaths among men and women, respectively, may be attributed to five modifiable risk factors, i.e., body mass index, current smoking, systolic blood pressure, non-high-density lipoprotein cholesterol, and diabetes mellitus. Among these modifiable risk factors, hypertension, dyslipidemia, and diabetes are considered as co-morbidities. With the advancement of medical research, new drugs and medical materials have become available for the treatment of CVD and its co-morbidities. For example, several novel therapies, such as sodium glucose co-transporter 2 inhibitors, have recently been shown to be of benefit when treating CVD in patients with type 2 diabetes, and in patients with heart failure. At the same time, these medical advances have also extended our average lifespan. For these reasons, there is a need for updated clinical studies in these fields, aiming to optimize the outcomes of patients with diabetes, hypertension, and cardiovascular diseases. No single mechanism can tell the whole story of CVD and its co-morbidities; therefore, continuing medical research is needed as the driver of medical advancement.

This Special Issue, "Diabetes, Hypertension and Cardiovascular Disease: Clinical Insights, Mechanisms and Pharmacotherapies", was designed as a forum for authors to share important findings from their medical research focusing on diabetes, hypertension, and other cardiovascular diseases, and for others to organize and review the essence of their recent research. The published articles cover a wide range of cardiovascular-related topics, ranging methodologically from plasma biomarkers, prognostic assessment, and big data analyses to the applications of Chinese medicine. These articles underscore some important issues that we have to face and deal with in daily practice.

For coronary artery disease, Hung et al. [3] review recent advances regarding epicardial coronary artery spasm-induced vasospastic angina, one of the very important causes of myocardial ischemia with no obstructive coronary arteries (INOCA). Local and systemic inflammation can be found in patients with vasospastic angina. Therefore, treatment strategies to decrease inflammation might be effective for these patients. However, the importance of correct diagnosis as the first step in achieving effective treatment cannot be overemphasized. In other words, it is important to identify the underlying cause of INOCA, especially in this era of primary coronary intervention, because inaccurate diagnosis can lead to insufficient treatment and poor prognosis, such as the development of heart failure [4].

In addition to the traditional evaluation of the association between biomarkers and coronary artery disease, Yang et al. [5] explore the use of three-dimensional anthropometric body surface scanning measurements. They show that these scanning measurements,

in combination with leptin, adiponectin and interleukin-6 levels, offer the best level of discrimination to identify individuals at risk of coronary artery disease.

Regarding peripheral artery disease, Onofrei et al. [6] evaluate the role of novel inflammatory markers in assessing the severity of peripheral artery disease. Their novel inflammatory markers include the neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and lymphocyte-to-C-reactive protein ratio. These three markers have important roles in treatment benefits and prognostic implications.

Obesity is suggested to have paradoxical survival benefits in patients with heart failure. Alrob et al. [7] identify an inverted U-shaped relationship between body mass index and left ventricular ejection fraction in patients with heart failure and reduced ejection fraction. Therefore, they propose the existence of an obesity paradox among patients with these conditions.

Regarding heart failure, Tsai et al. [8] use a large data collection from the Chang Gung Research Database to analyze the effects of heart rate reductions on the prognosis of patients with decompensated heart failure and reduced ejection fraction. A greater reduction in heart rate after hospital discharge is associated with a better prognosis. Hence, they suggest that achieving a target heart rate reduction leads to a lower prevalence of cardiovascular death, hospitalization for heart failure, and all-cause mortality.

For hypertension, Aursulesei Onofrei et al. [9] assess the prognostic value of the subendocardial viability ratio (SEVR), i.e., the Buckberg index, in patients with hypersensitivity. They describe how SEVR is associated with age, central and peripheral systolic blood pressure, heart rate, serum fibrinogen, and serum hemoglobin. The prognostic value of SEVR in hypertension is suggested by modulating the Framingham Risk Score values and Systemic Coronary Risk Evaluation risk values.

Addressing safety concerns about the use of idarucizumab for the reversal of the direct oral anticoagulation agent, dabigatran, Dai et al. [10] retrospectively analyze a cohort study based on electronic medical records from the Chang Gung Research Database in Taiwan. They suggest that idarucizumab can safely and effectively be used to reverse the anticoagulant effect of dabigatran. This result provides real-world evidence for the application of idarucizumab.

Joo et al. [11] report on the hypoglycemic effect of modified Gangsimtang, an herbal decoction, on a patient with type 2 diabetes mellitus who refused to undergo conventional therapies. No adverse events were reported over 200 days of follow-up.

To evaluate the efficacy and safety of the Compounded Danshen Dripping Pill (CDDP) with regard to blood viscosity in patients with type 2 diabetes mellitus, Wi et al. [12] systemically searched seven databases that used CDDP to treat type 2 diabetes mellitus. Although their meta-analysis shows that CDDP reduces blood viscosity in type 2 diabetes mellitus, further high-quality, well-designed studies are needed to provide more solid evidence for its future applications.

MicroRNA is found to play important roles in the pathophysiology of diabetic kidney disease. Lee et al. [13] examine the potential applications of urinary microRNA on diabetic kidney disease from the emerging experimental and clinical evidence. While the results of using urinary microRNA as a non-invasive disease marker to predict diabetic kidney disease appear promising, further experimental standardization and clinical verification trials are warranted to promote its application practically.

Glucagon-like peptide receptor agonists (GLP-1 RAs) are proven to reduce glucose levels in patients with type 2 diabetes; their evidence of safety and prognostic implications remain to be further evaluated in relation to different drugs in the same class. Hu and Tsai et al. [14] found that semaglutide is associated with better outcomes for heart failure and cardiovascular death in patients classed as non-diabetic obese, whereas liraglutide is associated with worse outcomes of heart failure in patients with diabetes with a reduced ejection fraction. The GLP-1 RAs could reduce macroalbuminuria but could not improve renal function. The GLP-1 RAs still provide benefits in patients with type 2 diabetes or obesity.

With the publication of this Special Issue, its readers will have an opportunity to find useful information for their future research interests, including some helpful solutions for the challenges they encounter in their own works.

**Conflicts of Interest:** The author declares no conflicts of interest.

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*Article*

# Subendocardial Viability Ratio Predictive Value for Cardiovascular Risk in Hypertensive Patients

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**Abstract:** *Background*: The subendocardial viability ratio (SEVR), also known as the Buckberg index, is a parameter of arterial stiffness with indirect prognostic value in assessing long-term cardiovascular risk. *Materials and Methods*: We conducted a prospective cohort study on 70 patients with uncomplicated hypertension admitted to a county medical reference hospital. We analyzed demographics, laboratory data, arterial stiffness parameters and cardiovascular risk scores (SCORE and Framingham risk scores) and aimed to identify paraclinical parameters associated with increased cardiovascular risk. *Results*: Of the arterial stiffness parameters, SEVR correlates statistically significantly with age, central and peripheral systolic blood pressure, as well as with heart rate. SEVR seems to have prognostic value among hypertensive patients by increasing the risk of major cardiovascular events assessed by SCORE and Framingham risk scores. SEVR correlates statistically significantly with serum fibrinogen ($p = 0.02$) and hemoglobin ($p = 0.046$). Between pulse wave velocity and lipid parameters ($p = 0.021$ for low-density lipoprotein cholesterol <LDL> and $p = 0.030$ for triglycerides) a statistically significant relationship was found for the study group. The augmentation index of the aorta also correlated with serum LDL-cholesterol ($p = 0.032$) and the hemoglobin levels ($p = 0.040$) of hypertensive patients. *Conclusions*: Age, abdominal circumference and Framingham score are independent predictors for SEVR in our study group, further highlighting the need for early therapeutic measures to control risk factors in this category of patients.

**Keywords:** hypertension; cardiovascular risk factors; arterial stiffness; Framingham score; atherosclerosis; pulse wave velocity; subendocardial viability ratio

## 1. Introduction

Hypertension is one of the most common cardiovascular risk factors encountered, and its prevalence continues to rise in the face of primary prevention measures. Based on the fact that age and increased blood pressure values are the main determinants of arterial stiffness [1], the identification of paraclinical parameters with an active role in assessing the long-term prognosis of these patients is a research direction of the academic community in this field.

In addition to hypertension, diabetes mellitus, dyslipidemia, smoking and chronic kidney disease are involved in pathophysiological processes at the level of the vascular wall that over time lead to decreased compliance and elasticity due to changes in the extracellular matrix components at that level [2]. Arterial stiffness causes increased pulse pressure, left ventricle afterload and decreased diastolic blood pressure (BP) [3,4]. Over

time, these hemodynamic changes constitute the pathophysiological basis of myocardial ischemia by increasing myocardial oxygen requirements, decreasing coronary perfusion and thus decreasing oxygen supply to the myocardium [5–7]. The pathophysiological implications of arterial stiffness on the development of renal dysfunction have been extensively researched, with recent data suggesting a statistically significant and independent link between the presence of arterial stiffness and reduced glomerular filtration rate in hypertensive patients [8].

The subendocardial viability ratio (SEVR), also known as the Buckberg index [9], is an arterial stiffness parameter correlated with coronary flow reserve which makes it a useful parameter in assessing coronary microvascular circulation in hypertensive patients [10,11]. Recent data from the literature attest to the existence of a gender-differentiated SEVR, with women's risk of developing CAD being mainly secondary to an accelerated drop in aortic diastolic pressure determining myocardial perfusion impairment [12].

In the context of the continuous increase in the prevalence of cardiovascular risk factors and the continuous development of diagnostic and treatment methods, the identification of parameters with a prognostic role in this category of patients is useful in guiding therapeutic management [13].

The aim of this study was to identify SEVR's role in the assessment of long-term cardiovascular risk in hypertensive patients as well as its determinants in the study group, with the aim of preventing potentially fatal acute cardiovascular events. We aim to use different risk scores that were validated on different populations for a comprehensive overview.

## 2. Materials and Methods

### 2.1. Study Design and Population

We conducted a prospective, single-center clinical study including 70 patients with uncomplicated hypertension, admitted to the Cardiology Department of "St. Spiridon" Hospital. Of the 70 patients, we excluded 13 patients in whom arterial stiffness parameters could not be recorded, as well as one patient with incomplete data regarding treatment or laboratory data. The final study group included 56 hypertensive patients who were followed-up for a period of 12 months.

We included patients over the age of 18 years old with a diagnosis of essential arterial hypertension and who have given their written consent to participate in the study. The exclusion criteria were the refusal of patients to participate in the study, a personal history of arrythmias (including cardiac pacing), and diagnosis of cardiac ischemic disease with-/without interventional or surgical revascularization, stroke, cancer, as well as patients in whom arterial stiffness parameters could not be assessed.

### 2.2. Measurements

We analyzed a variety of parameters such as demographics (age, gender), cardiovascular (CV) risk factors, anthropometric parameters, vital signs (systolic blood pressure <SBP, mmHg>, diastolic blood pressure <DBP, mmHg> and heart rate <beats per minute, bpm>) as well as arterial stiffness parameters. The diagnosis of hypertension has been established according to current guidelines [13].

The laboratory testing consisted of lipid profile (total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, tryglicerides), serum glucose, uric acid, renal function parameters (creatinine, urea), fibrinogen and hemoleucogram. The results were presented according to the International System of Units.

Cardiovascular risk scores were calculated based on the CV risk factors obtained from the medical interview, physical examination and biochemical tests. Two major CV risk scores were computed: the Framingham risk score [14,15] and SCORE (Systematic Coronary Risk Evaluation Project) risk [16,17].

### 2.3. Atherosclerosis Evaluation Using the Artheriograph Device

Using an Arteriograph, several parameters of AS were determined noninvasively, and the focus was on pulse wave velocity (PWV), the augmentation index of the aorta (AixAo), systolic area under the pulse wave curve (SAI), and the diastolic area below the pulse wave curve (DAI). SEVR was determined using the ratio of the areas of the systolic and diastolic portions below the aortic pulse wave curve and denoted as systolic area index (SAI) and diastolic area index (DAI), respectively. Knowing that SAI is calculated as the product of mean systolic LV pressure and systole duration and DAI as the product of the difference between mean aortic diastolic pressure and mean diastolic LV pressure and diastole duration, we rewrote the calculation formula as follows [18] (Figure 1):

$$\text{SEVR} = \frac{(\textbf{mean aortic diastolic pressure} - \textbf{mean diastolic LV pressure}) \times \textbf{diastole duration}}{\textbf{mean systolic LV pressure} \times \textbf{systole duration}}$$
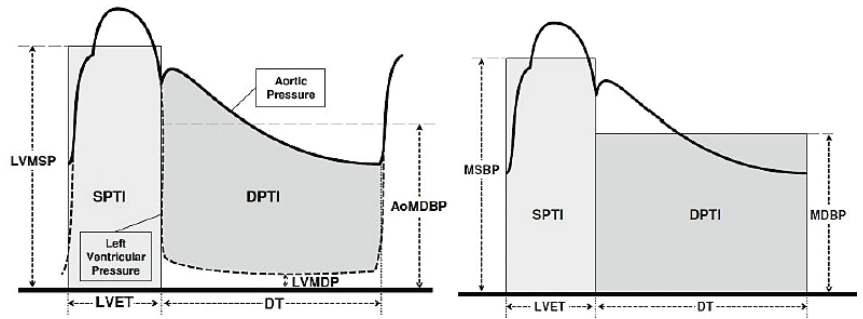


**Figure 1.** SEVR determinants (MSBP: mean blood pressure, SPTI: systolic pressure time index, DPTI: diastolic pressure time index, LVET: LV ejection duration, LVMDP: mean diastolic pressure in LV, LVMSP: mean systolic pressure in LV, DT: diastole duration) (adapted after [18]).

The parameter measurement protocol via the Arteriograph device involved the following steps: (1) recording general patient data (name, date of birth, weight, height, arm circumference and abdominal circumference, the distance between the sternal notch and the upper edge of the pubic symphysis, without following the abdominal relief); (2) locating the area of maximum pulsatility of the brachial artery, with the positioning of the cuff of the device at this level; (3) the initiation of measurements, with the patient lying on his back and tracking the recording of pulse waves on the monitor to observe the morphology of the route; and (4) the interpretation of the results. In addition, before and during the recording the following measures were observed: the examination was performed in a quiet environment, and during patient mobilization, the avoidance of speech during the measurement was encouraged. When the "white coat" effect was suspected regarding hypertension, an attempt was made to reassure the patient and repeat the measurement. Smoking and coffee consumption were suppressed at least 3 h before the examination, copious meals were avoided during this period, as was the administration of nitrates. Alcohol consumption was prohibited for an period of 10 h before the administration.

### 2.4. Statistical Analysis

A statistical analysis was performed using SPSS statistics software (Statistical Package for the Social Sciences version 23 for Windows; SPSS Inc., Chicago, IL, USA). An initial descriptive analysis of the variables was performed for the continuous type variables calculating the mean, the median, minimum and maximum values, quartiles, and standard deviation. Skewness (measuring the symmetry of the variables with respect to the mean value) and kurtosis (flattening coefficient) were determined to assess the normal distribution of continuous variables by using the Shapiro–Wilk test. All numerical variables had a normal distribution and were presented as means ± standard deviation.

To compare the mean values between two groups of continuous values in order to determine the statistically significant differences, the *t*-test (independent *t* test) and ANOVA (one way analysis of variance) was used. Pearson (for continuous variables) and Spearman (for categorical variables) correlation coefficients were used to assess the presence of correlations between the studied variables. For the subsequent analysis of the relationship between the variables that met the statistical threshold

for the realized correlations, a simple linear regression was performed, and as well as by selecting several independent variables that influence a dependent variable, simple linear regression was extended to multiple regression. A *p*-value < 0.05 was considered statistically significant.

*2.5. Ethics*

The study protocol was approved by the local Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy Iași and of "St. Spiridon" Clinical Emergency Hospital, and was conducted in accordance with the terms of the Helsinki Declaration. All participants signed an informed written consent before enrollment.

### 3. Results

We enrolled 56 patients diagnosed with uncomplicated essential hypertension who had been evaluated in an integrative and multidisciplinary approach. The study group included predominantly male patients (62.5%), with an average age of 67.62 ± 9.78 years.

In addition to demographics, the statistical analysis also included vital parameters. Table 1 lists the parameters associated with cardio-metabolic profiles according to gender. In our study, SBP had a mean value of 143.23 ± 28.81 mmHg in the whole group, with no statistically significant gender differences (*p* = 0.538). DBP had an average value of 78.96 ± 16.44 mmHg, and the mean blood pressure (MBP) averaged 100.42 ± 19.7 mmHg. Pulse pressure (PP) had had an average value of 64.26 ± 17.71 mmHg, with a mean value slightly higher in women (67.23 ± 13.81 mmHg) compared to men (62.48 ± 19.65 mmHg) (Table 1).

**Table 1.** Gender distribution of systolic blood pressure and pulse pressure.

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean Lower Bound–Upper Bound | | Min | Max | *p* |
|---|---|---|---|---|---|---|---|---|---|
| Systolic blood pressure (mmHg) | | | | | | | | | |
| Females | 21 | 145.524 | 26.0684 | 5.6886 | 133.658 | 157.390 | 102.0 | 219.0 | |
| Males | 35 | 141.857 | 30.6231 | 5.1762 | 131.338 | 152.377 | 103.0 | 224.0 | 0.538 |
| Total | 56 | 143.232 | 28.8103 | 3.8499 | 135.517 | 150.948 | 102.0 | 224.0 | |
| Pulse pressure (mmHg) | | | | | | | | | |
| Females | 21 | 67.238 | 13.8163 | 3.0150 | 60.949 | 73.527 | 43.0 | 98.0 | |
| Males | 35 | 62.486 | 19.6594 | 3.3230 | 55.732 | 69.239 | 36.0 | 110.0 | 0.305 |
| Total | 56 | 64.268 | 17.7123 | 2.3669 | 59.524 | 69.011 | 36.0 | 110.0 | |

The mean heart rate was 67.16 ± 11.11 beats per minute (bpm) in the whole group (statistically analyzed), without identifying statistically significant differences between genders (67.33 ± 10.94 bpm vs. 67.05 ± 11.26 bpm). Regarding anthropometric parameters, special attention was paid to the value of abdominal circumference, which had an average value above the upper limit of the normal range of values for both females and males (109.88 ± 12.605 vs. 101.36 ± 11.95 cm).

In addition to demographic and hemodynamic data, the duration of hypertension was also included in the statistical analysis. Thus, three patients (7.3%) had hypertension of up to 1 year old, 10 patients (24.4%) had hypertension from 1 to 5 years since diagnosis, 12 patients (29.3%) had hypertension from 5 to 10 years since diagnosis, and 16 cases (39%) had hypertension for more than 10 years since diagnosis. In 15 patients it was not possible to determine the duration of hypertension from the medical history or existing medical documents.

We evaluated a variety of biological parameters, both hematological and biochemical, to outline the metabolic profile of the 56 patients enrolled in the study (Table 2). Thus, mean serum glucose (125.20 ± 44.35 mg/dL), low-density lipoprotein (LDL), cholesterol (121.67 ± 42.18 mg/dL) and uric acid (5.61 ± 1.62 mg/dL) levels were above the upper limit of the normal range. These parameters also represent risk factors for increased morbidity due to acute cardiovascular events in hypertensive patients who frequently associate with dyslipidemia, diabetes mellitus or changes in purine metabolism. In terms of renal function parameters, although mean serum urea and creatinine levels were within the normal range, eGFR was associated with low mean values (82.80 ± 23.89).

**Table 2.** Description of the analyzed biochemical and hematological parameters.

| Biological Parameters | Minimum | Maximum | Mean Value | Standard Deviation |
|---|---|---|---|---|
| Hemoglobin (g%) | 10.30 | 18.00 | 13.5091 | 1.65678 |
| Hematocrit (%) | 33.00 | 53.10 | 40.4241 | 4.41400 |
| Fasting glucose, mg/dL | 62 | 287 | 125.20 | 44.357 |
| Total cholesterol, mg/dL | 105 | 296 | 195.67 | 45.622 |
| HDL-cholesterol, mg/dL | 14 | 112 | 46.98 | 15.673 |
| LDL-cholesterol, mg/dL | 55 | 205 | 121.67 | 42.186 |
| Triglycerides, mg/dL | 57 | 438 | 140.13 | 79.310 |
| Uric acid, mg/dL | 3.30 | 9.70 | 5.6116 | 1.62501 |
| Fibrinogen, mg/dL | 270.0 | 490.0 | 389.231 | 59.3743 |
| Urea, mg/dL | 16.00 | 75.00 | 40.6364 | 13.24465 |
| Serum creatinine, mg/dL | 0.57 | 1.97 | 0.9462 | 0.28369 |
| eGFR (mL/min/1.73 m$^2$) | 36 | 135 | 82.80 | 23.896 |

HDL: high-density lipoprotein; LDL: low-density lipoprotein; eGFR: estimated glomerular filtration rate.

Regarding the parameters of arterial stiffness we used the oscillometric analysis of the pressure curves recorded at the level of the brachial artery by the Arteriograph device (Table 3). We evaluated the PWV at the central level (PWVao) whose average value was $9.75 \pm 1.74$ m/s, the AIx with a mean value of $32.82 \pm 14.02\%$, the SEVR with an associated mean value of $107.87 \pm 28.14\%$, and diastolic reflection area (DRA), with a mean value of $40.81 \pm 13.22$.

**Table 3.** Characteristics of arterial stiffness parameters.

| | PWVao [m/s] | AIx Aortic [%] | SEVR % | DRA |
|---|---|---|---|---|
| Mean | 9.757 | 32.823 | 107.8725 | 40.812 |
| Median | 9.750 | 32.050 | 108.3300 | 38.850 |
| Standard deviation | 1.7434 | 14.0231 | 28.14657 | 13.2270 |
| Minimum | 5.8 | 6.5 | 26.74 | 10.4 |
| Maximum | 14.1 | 63.2 | 161.78 | 75.3 |
| Percentile | | | | |
| 25 | 8.325 | 23.700 | 91.0225 | 32.875 |
| 50 | 9.750 | 32.050 | 108.3300 | 38.850 |
| 75 | 10.900 | 43.800 | 125.1025 | 48.900 |

PWVao: pulse wave velocity at the central level; AIx: augmentation index; SEVR: subendocardial viability index; DRA: diastolic reflection area.

In addition to a descriptive statistical analysis, various statistical correlations were made between SEVR and biological parameters, other arterial stiffness parameters, and risk scores as shown in Table 4 and Figures 2–4. Between SEVR and age there is an inverse, statistically significant relationship ($p = 0.005$, r = $-0.367$) which highlights the decreasing trend of SEVR with age in our group. A simple linear regression was calculated to observe the influence of age on SEVR. A significant regression equation was highlighted (F (1.54) = 8.428, $p = 0.005$), with an R$^2$ = 0.135. According to the analysis, SEVR associates a decrease of $-1057$ for an increase of age by one unit in the studied cases.

**Table 4.** Correlations between SEVR and hematological, biochemical parameters, or arterial stiffness parameters.

| | SEVR | | PWVao [m/s] | | AIx Aortic [%] | |
|---|---|---|---|---|---|---|
| | **r** | *p* | **r** | *p* | **r** | *p* |
| **Biochemical parameters** | | | | | | |
| Fasting glucose (mg/dL) | 0.02 | 0.87 | 0.192 | 0.159 | −0.008 | 0.956 |
| Total cholesterol (mg/dL) | 0.02 | 0.84 | 0.245 | 0.079 | 0.306 | 0.027 |
| HDL-cholesterol (mg/dL) | 0.11 | 0.41 | −0.254 | 0.082 | 0.114 | 0.439 |
| LDL-cholesterol (mg/dL) | −0.07 | 0.59 | 0.330 | 0.021 | 0.307 | 0.032 |
| Triglycerides (mg/dL) | 0.16 | 0.23 | 0.301 | 0.030 | 0.081 | 0.569 |

**Table 4.** *Cont.*

| | SEVR | | PWVao [m/s] | | AIx Aortic [%] | |
|---|---|---|---|---|---|---|
| | r | p | r | p | r | p |
| Fibrinogen (mg/dL) | 0.455 | 0.02 | 0.346 | 0.083 | −0.260 | 0.199 |
| Serum urea (mg/dL) | −0.09 | 0.49 | 0.160 | 0.244 | 0.262 | 0.053 |
| Serum creatinine (mg/dL) | 0.04 | 0.77 | 0.014 | 0.917 | −0.048 | 0.728 |
| Uric acid (mg/dL) | −0.01 | 0.96 | 0.193 | 0.290 | −0.107 | 0.561 |
| Hemoglobin (g%) | 0.270 | 0.046 | −0.083 | 0.546 | −0.277 | 0.040 |
| Hematocrit (%) | 0.211 | 0.125 | −0.085 | 0.539 | −0.249 | 0.069 |
| **Arterial stiffness parameters** | | | | | | |
| Central SBP (mmHg) | −0.304 | 0.023 | 0.270 | 0.044 | 0.293 | 0.029 |
| Peripheral SBP (mmHg) | −0.350 | 0.008 | 0.242 | 0.073 | −0.010 | 0.942 |
| DBP (mmHg) | −0.154 | 0.256 | 0.196 | 0.147 | −0.118 | 0.388 |
| MBP (mmHg) | −0.258 | 0.055 | 0.230 | 0.088 | −0.070 | 0.608 |
| PP (mmHg) | −0.426 | 0.001 | 0.211 | 0.119 | 0.093 | 0.495 |
| Heart rate, bpm | −0.301 | 0.024 | 0.203 | 0.133 | −0.478 | <0.001 |

r: Pearson Correlation; HDL: high-density lipoprotein; LDL: low-density lipoprotein; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure; PP: pulse pressure; bpm: beats per minute.



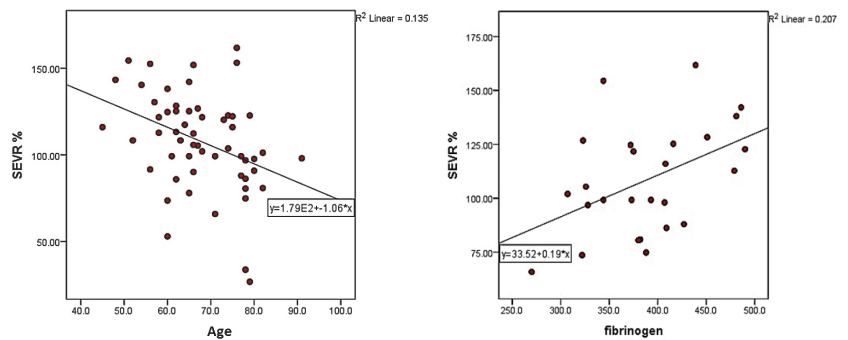**Figure 2.** Correlation of SEVR values with age and serum fibrinogen (SEVR: subendocardial viability ratio).
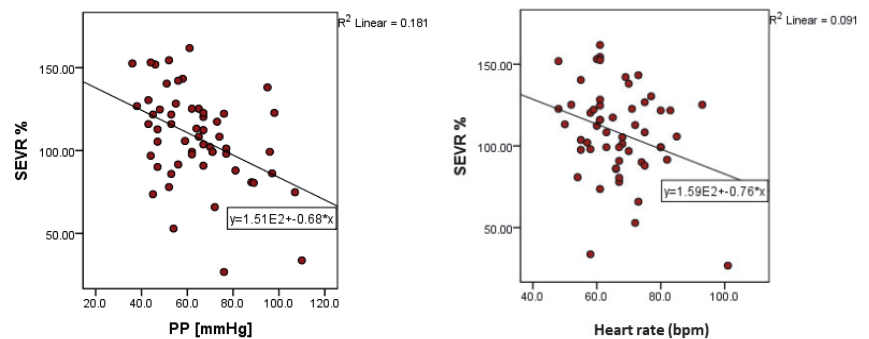


**Figure 3.** Correlation of SEVR values with pulse pressure and heart rate (SEVR: subendocardial viability ratio; PP: pulse pressure).
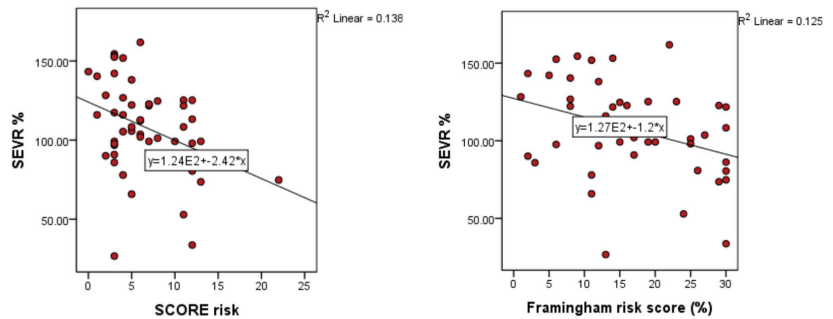
**Figure 4.** Correlation of SEVR values with SCORE risk values and Framingham risk score values (SEVR: subendocardial viability ratio).

SEVR was also analyzed in relation to the hematological and biochemical parameters evaluated on enrolment in the study. Two statistically significant positive correlations were highlighted, with hemoglobin levels ($p = 0.046$, r = 0.27) and with fibrinogen levels (r = 0.455 at $p = 0.02$). The pathological values of fibrinogen were considered to be over 400 mg/dl, and after the application of the *t*-test there was a significant difference between patients with normal fibrinogen values (mean = 100.33 ± 23.98) and those with pathological values (mean = 119.93 ± 23.20), *t* (24) = −2.086, $p = 0.048$.

To further analyze the relationship of SEVR with fibrinogen, a simple linear regression was calculated. A significant regression equation was highlighted (F (1.24) = 6.257, $p = 0.02$), with an $R^2 = 0.207$. According to the analysis, the SEVR recorded an increase of 0.193 for an increase in fibrinogen by one unit in the cases studied.

Furthermore, a series of negative correlations were observed between SEVR and central SBP ($p = 0.023$, r = −0.304), peripheral SBP ($p = 0.008$, r = −0.350), PP ($p = 0.001$, r = −0.426), and heart rate ($p = 0.024$, r = −0.301). A multiple linear stepwise regression was calculated to observe the influence of the values of the different parameters of blood pressure and HR on SEVR. A significant regression equation was highlighted (F (2.53) = 9.78, $p < 0.001$), with an $R^2 = 0.270$. According to the analysis, SEVR records a decrease of −0.672 for an increase in pulse pressure by one unit, and by −0.753 for an increase in HR by one unit. Both PP ($p < 0.01$) and heart rate ($p = 0.01$) are statistically significant independent predictors of SEVR.

For the patients in the studied group, the SCORE and Framingham risk scores were also calculated. These correlated negatively with the SEVR values, for the Framingham score registering a $p = 0.014$, r = −0.353, and for the SCORE $p = 0.007$, r = −0.371. To further analyze the relationship of SEVR with SCORE, a simple linear regression was calculated. A significant regression equation was highlighted (F (1.50) = 7.995, $p = 0.07$), with an $R^2 = 0.138$. According to the analysis, SEVR registers a decrease of −2.41 for an increase of SCORE by one unit. To analyze the relationship of SEVR with Framingham risk score, a simple linear regression was calculated. A significant regression equation was highlighted with an $R^2 = 0.125$.

In an attempt to determine the variables that independently influence SEVR, a series of models were made by multiple linear regression. The equation with the highest value of adjusted $R^2$ includes as independent predictors the abdominal circumference (β = −0.623, $p < 0.001$), age (β = −0.213, $p = 0.031$), and Framingham score (β = −0.540, $p < 0.001$), all three reaching the threshold of statistical significance ($p < 0.05$), with adjusted model $R^2 = 0.949$, $p < 0.001$. A significant regression equation was highlighted (F (3.8) = 69.541, $p < 0.001$), with an $R^2 = 0.963$. According to the analysis the SEVR decreased by −31,396 for patients with abdominal obesity, by −1513 to increase the Framingham score by one unit, and by 0.577 with the increase of age by one unit in the cases studied. Abdominal obesity, Framingham score and age are statistically significant independent predictors of SEVR.

When only continuous variables that correlated with SEVR (central SBP, PP, HR, age, fibrinogen value, Hb, Framingham and SCORE risk scores) were introduced into the analysis, a model was obtained with: SCORE (β = −0.441, $p = 0.005$), fibrinogen values (β = 0.428, $p = 0.004$), and Hb (β = 0.382, $p = 0.013$), all three reaching the threshold of statistical significance ($p < 0.05$), with $R^2 = 0.670$ and adjusted $R^2 = 0.618$.

A significant regression equation was highlighted (F (3.19) = 12.845, $p < 0.001$), with an $R^2 = 0.670$. According to the analysis, SEVR records a decrease of −2403 for each increase in SCORE by one unit, an increase of 0.182 for an increase in fibrinogen by one unit, and an increase of 6675 with an

increase in Hb by one unit in the studied cases. SCORE, fibrinogen and Hb were thus determined as statistically significant independent predictors of SEVR.

## 4. Discussion

Hypertension has a dual role as a cardiovascular risk factor and as a disease with an increasing prevalence in the context of accelerated global industrialization. It is also responsible for the occurrence of potentially fatal acute cardiovascular events in the absence of a personalized and integrative management of each individual patient. Identification of paraclinical parameters associated with increased cardiovascular risk is essential, having a dual role: prognostic and therapeutic.

In our study, we demonstrated that SERV correlates with age, fibrinogen and hemoglobin serum levels, as well as with various arterial stiffness parameters such as pulse pressure, central, and peripheral SBP.

SEVR is influenced by several factors, including demographics. In our study, we demonstrated that SEVR correlates negatively with age. In addition, based on the concept that age is an element of the Framingham score, we emphasized the impact of age on SEVR by obtaining a statistically significant correlation with this cardiovascular risk score ($p = 0.014$). SEVR modulates the long-term prognosis of patients with hypertension via associated arterial stiffness, which is an indirect marker of aging or the onset/progression of atherosclerotic processes [19]. Laugesen et al. [20] demonstrated that women with diabetes have a lower SEVR than men with ($p < 0.01$) or without diabetes ($p < 0.001$) or even women without diabetes ($p < 0.001$), with statistically significant correlations after adjusting for various cofounders such as age, BP, HR or smoking. In a similar study published recently, Kaname et al. [12] highlighted that aortic diastolic pressure decay underlies the SEVR differences between genders. Thus, women have a higher aortic diastolic pressure decay index compared to men, even after adjusting results for age, dyslipidaemia or diabetes, as well as a lower SEVR value ($p < 0.001$). Saito et al. [21] observed lower SEVR values in elderly patients with a BMI outside the normal range, increased heart rate, dyslipidaemia and increased serum glucose levels. Of the risk factors mentioned above, only age and heart rate have been shown to be independent predictors of SEVR. Changes in the vascular walls that occur with advancing age contribute to hypertension. Ma et al. [22] demonstrated through a statistical regression model that aortic and radial SEVR changes in the elderly are similar, this demographic parameter being an independent predictor in the study population [23]. Increasing arterial stiffness causes decreasing SEVR, the correlation with age being negative. The same group of researchers reported different results according to gender and age decade. Thus, while in male patients, SEVR values decreased in the third to fifth decades and then increased starting in the sixth decade, in females the increase in values up to the fifth decade was followed by a decrease starting in the sixth decade most likely secondary to the onset of menopause and its effect on arterial compliance [22,24,25]. A recently published clinical study correlates reduced SEVR and ankle-brachial index values with frequent systemic atherosclerotic disease in the elderly [26]. The different values of aortic and radial SEVR can also be explained in terms of age-associated vascular wall changes that occur more frequently in the aorta compared to peripheral arteries [27].

SEVR is an indirect parameter of the myocardial oxygen supply and demand [10]. The reduction in diastolic aortic pressure consequently causes a reduction in myocardial perfusion, thus highlighting the high susceptibility of the myocardium to various factors that infuse oxygen supply such as hypertension [28–30]. Between SEVR and cardiovascular risk there is an inversely proportional relationship, with decreasing SEVR values being associated with increased cardiovascular risk and worsening prognosis predominantly in patients with diabetes or chronic kidney disease [20,31,32]. Patients with chronic kidney disease and low SEVR have a high risk of myocardial oxygen demand impairment [33]. In addition to glomerular filtration rate, SEVR modulates serum cystatin C levels even in patients without kidney impairment [34].

Tsiachris et al. [10] investigated the role of SEVR as a predictor of coronary microcirculation in hypertensive patients and observed a 24.5% decrease in this parameter in hypertensive patients with low coronary flow reserve ($p = 0.0002$). The same study also highlighted the independent predictive role of age, left ventricular mass index and diastolic BP alongside SEVR for coronary flow reserve.

SEVR is an indirect marker of the pathophysiological burden of metabolic syndrome on arterial function. The pathophysiological rationale lies in the pathophysiological effect of the metabolic syndrome on subclinical vascular damage and increased arterial stiffness [35–37]. In our study, no statistically significant correlations were observed between SEVR and components of the lipid profile (total cholesterol and triglycerides), but clinically and prognostically significant results were recorded for abdominal circumference, which was found to be an independent predictor ($p = 0.031$). Although no statistically significant correlations were found, changes in lipid, carbohydrate and uric acid profile

parameters have a similar negative impact to classical cardiovascular risk factors, contributing to the development or evolution of atherosclerotic processes, justifying changes in paraclinical parameters of arterial stiffness. Jekell et al. [31] also concluded that in hypertensive patients without diabetes mellitus, SEVR does not correlate with serum HDL-cholesterol levels or insulin resistance markers.

In analyzing our group of patients we demonstrated that patients with abdominal obesity had a 31.39 lower SEVR compared to the other patients. Our results are consistent with data presented by similar clinical studies in the literature. In a recent study, a group of investigators, Tocci et al. [38], demonstrated in a cohort of adolescents that overweight patients have reduced SEVR values (114.4 ± 25.9% vs. 132.2 ± 22.0% respectively, with a *p* value of 0.038) compared to normal-weight patients, similar to carotid-femoral pulse wave velocity and aortic systolic blood pressure (*p* = 0.043). Marčun-Varda et al. [39] analyzed a cohort of pediatric patients with different cardiovascular risk factors and observed a potential link with cardiovascular risk through correlations with age, heart rate and mean central BP, but further studies in the field are needed to confirm this. Although the value of SEVR as a predictor of decreased myocardial viability in overweight patients has not been demonstrated, its decrease secondary to consecutive myocardial work and aortic systolic pressure augmentation is a direction for future research in the field [40].

The correlation between arterial stiffness, SEVR and the cardio-metabolic risk factors have been investigated by Fantin et al. [41] in a recent study in which 55 patients with metabolic syndrome were enrolled. The group of investigators demonstrated that the presence of metabolic syndrome correlates with reduced SEVR values (*p* = 0.012), even after adjusting multivariate regression for different cofactors such as age, gender or mean arterial blood pressure (*p* = 0.040). The number of metabolic syndrome components also influences the evolution of SEVR, its values decreasing with increasing number of metabolic syndrome elements (*p* = 0.005).

Among the laboratory parameters included in the statistical analysis, between fibrinogen (*p* = 0.02), hemoglobin (*p* = 0.046) and SEVR there are statistically significant correlations for our study group. There is a complex pathophysiological relationship between anemia and cardiovascular risk, mediated in many cases by the presence of chronic kidney disease [42]. Serum blood glucose is an important cardiovascular risk factor with therapeutic and prognostic implications, although SEVR, PWV and AIx were not statistically significantly correlated with glycemia in our study group. The lack of statistically significant correlations can be explained by the normal values of the velocities obtained in the patients enrolled in the present study. Clinical studies, however, highlight the presence of altered values of arterial stiffness parameters in diabetic patients. Di Pino et al. [43] have shown that patients with prediabetes and high glomerular filtration rate values have increased augmentation pressure and AIx values and reduced SEVR values (*p* < 0.05). In patients with type 1 diabetes mellitus, reduced SEVR values correlate independently and negatively with the presence and degree of microalbuminuria and are a superior predictor of PWV in assessing the albumin excretion rate [44]. The duration of diabetes also modulates the SEVR value, which is reduced in women with type 2 diabetes diagnosed no more than 5 years ago [20].

Ekart et al. [45] demonstrated that SEVR is dependent on serum hemoglobin and troponin levels in a cohort of 91 patients with kidney disease (non-dialysis). In addition, the subgroup of patients with anemia was characterized by a higher serum creatinine level, higher blood pressure values, and a lower SEVR value than the cases without anemia. In a more recent clinical trial, the same group of investigators demonstrated that chronic kidney disease patients with an SEVR of less than 130% were associated with a 16-fold increased risk of fatal cardiovascular events compared to patients with an SEVR greater than 130% (*p* = 0.004) [46]. Not only do low eGFR values correlate with SEVR, but high ones do as well, with the main determinants of SEVR in the prediabetic population being SBP, eGFR and insulin resistance as major determinants of arterial stiffness [43]. Based on the concept that a significant percentage of hypertensive patients are associated with peripheral arterial disease, the analysis of paraclinical parameters of arterial stiffness has prognostic value. The identification of a reduced ankle-brachial index value correlates with an increased PWV value, but not with a decreased SEVR [47].

The evolution of SEVR in relation to age, oxygen saturation and serum hemoglobin level was studied in a group of 41 hospitalized heart failure patients in order to identify predictors involved in the risk of rehospitalization at 30 days [48]. Clinical improvement resulted in statistically significant improvement in SEVR interpreted both in isolation and after correcting for serum hemoglobin, leading to the conclusion that the administration of medical therapy (predominantly diuretics) induces improvement in arterial perfusion and subendocardial perfusion in geriatric patients. The variation of SEVR as a function of serum hemoglobin and arterial oxygen saturation was not only

observed in hypertensive patients but also in those with orthostatic hypotension in whom SEVR values were lower ($p = 0.05$) and PWVAo higher ($p = 0.042$) [49].

In addition to demographic parameters and laboratory data, SEVR also correlates with arterial stiffness parameters. In our statistically analyzed group, we demonstrated that statistically significant correlations exist between SEVR and central SBP, peripheral SBP, and heart rate. Also, PP and frequency were found to be independent predictors of SEVR in multivariate regression, aspects correlating with the data presented in the literature. We also demonstrated that central SBP correlates statistically significantly with PWV ($p = 0.044$) and Aix ($p = 0.029$).

Anyfanti et al. [50] also emphasize the usefulness of SEVR in assessing microvascular coronary perfusion as well as its variability according to blood pressure phenotype. SEVR varies according to blood pressure phenotype, and the group of investigators observed that normotensive patients have higher SEVR values compared to those with masked hypertension, white-coat hypertension or true hypertension ($p = 0.017$). In addition, central SBP, peripheral SBP and the total arterial compliance index were found to be predictors in univariate statistical analysis, with value retained after adjusting for heart rate. Pulse pressure also influences SEVR values in elderly hypertensive patients. Chemla et al. [25] concluded that between SEVR and diastolic time over systolic time ratio there is a positive linear correlation for a given cut-off value of this ratio. SEVR is associated with lower hypertension in patients with PP over 60 mmHg compared to those with normal PP values.

Our study has several limitations due to the relatively small number of cases analyzed. We excluded patients from the study in whom arterial stiffness parameters could not be obtained or in whom the observation chart did not contain all the parameters necessary for statistical analysis.

## 5. Conclusions

Our results support the notion that the assessment of SEVR in patients with hypertension has prognostic value, being a useful pillar in the assessment of long-term cardiovascular risk by modulating SCORE and Framingham risk scores. Age, serum fibrinogen level, haemoglobin, heart rate and central and peripheral SBP are parameters that correlate statistically significantly with SEVR, but independent predictive value in multivariate statistical analysis was demonstrated only for age, abdominal circumference and Framingham risk score.

The value of SEVR as an index of long-term cardiovascular risk is even greater, as it is associated with a diversity of parameters, many of which are cardiovascular risk factors per se or have a defining role in increasing the cardiovascular risk of morbidity and mortality. These results raise the necessity of applying early specific therapeutic measures to control the CV risk factors in this group of patients.

**Author Contributions:** All authors contributed equally to this work; B.A. and A.C. (Adrian Crisan). performed the statistical analysis; V.A.O., A.C. (Alexandr Ceasovschih), C.M.S.H. and O.M. wrote the paper; D.T.M.M., C.A.A. and R.C.A. helped revise the language; M.R. and C.C. review editing; V.A.O. and F.M. selected the figures and revised the final script. All authors have read and agreed to the published version of the manuscript.

**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy Iași and of "St. Spiridon" Clinical Emergency Hospital (number 5/06.01.2017).

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Conflicts of Interest:** The authors declare that they have no conflict of interest.

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*Article*

# Obesity Paradox among Heart Failure with Reduced Ejection Fraction Patients: A Retrospective Cohort Study

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**Abstract:** *Background and Objectives:* There is consensus on the negative effects of obesity on the development of heart failure. However, several studies have suggested that obesity may have paradoxical survival benefits in heart failure patients. Therefore, the aim of this study is to investigate whether the obesity paradox exists in heart failure with reduced ejection fraction (HFrEF) patients in Jordan. Materials and *Methods*: In this retrospective cohort study, data were retrieved from electronic hospital records of heart failure patients admitted to King Abdullah University Hospital between January 2010 and January 2020. Patients were divided into five BMI (kg/m$^2$) subgroups: (1) Less than 25.0, (2) Overweight 25.0–29.9, (3) Obese Class I 30.0–34.9, (4) Obese Class II 35.0–39.9, and (5) Obese Class III ≥40.0. Changes in patients' clinical and echocardiographic parameters over one year were analyzed. *Results*: Data of a total of 297 patients were analyzed to determine the effect of obesity on heart failure. The mean age was $64.6 \pm 12.4$ years, and most patients (65.7%) were male. Among several co-morbidities, diabetes mellitus and hypertension were the most common and were present in 81.8% and 81.1% of patients, respectively. Over all patients, there was no significant change in EF after 1 year compared to baseline. However, only patients in the Obese Class I group had a statistically significant improvement in EF of $38.0 \pm 9.81\%$ vs. $34.8 \pm 6.35\%$ ($p = 0.004$) after 1 year. Importantly, among non-diabetic individuals, only Obese Class I patients had a significant ($p < 0.001$) increase in EF after 1 year compared to other BMI subgroups, a feature that was not observed among patients with diabetes. On the other hand, only Obese Class I patients with hypertension had a significant improvement ($p < 0.05$) in EF after 1 year compared to other BMI subgroups, a feature that was not observed among patients without hypertension. *Conclusions*: Our study demonstrates an inverted U-shaped relationship between BMI and EF such that patients with mild obesity (i.e., Obese Class I) had significant improvement in EF compared to those having a lower and higher BMI. We, therefore, suggest the existence of the obesity paradox among HFrEF patients in Jordan.

**Keywords:** obesity paradox; heart failure; body mass index; diabetes; ejection fraction

## 1. Introduction

Obesity has reached pandemic proportions, and it is estimated that more than 39% of the global population is either overweight or obese [1,2]. Obesity is a well-established major risk factor for the development of heart failure (HF) [3,4]. Obesity has independent adverse effects on cardiac structure and function [5]. The Framingham Heart Study has shown that for every unit increase in body mass index (BMI), the incidence of HF increases by 5% and 7% in men and women, respectively [3]. In addition, a recent causal genetic

analysis suggests that obesity should be recognized as a causal factor for the development of HF [6]. This study shows that high levels of BMI causally increase the risk of incidence and mortality of HF. There has been increasing concern since, according to recent estimates, the prevalence of overweight and obesity in Jordan among adults is high, at 69.6% and 35.5%, respectively [7].

Although obesity is a risk factor for HF, over the last two decades, several studies and meta-analyses have shown better survival among obese patients with chronic HF in comparison to lean healthy or underweight patients; this is termed the "obesity paradox" [8–13]. This improvement in survival was associated with significantly higher left ventricular ejection fraction (LVEF) in overweight and obese subjects compared to underweight or healthy subjects, especially in patients with reduced ejection fraction (EF) [13–15]. Interestingly, the study that proposed a causal relationship between obesity and HF showed evidence of the obesity paradox using a case-only study design, in which obese HF patients had improved survival compared to HF patients with normal weight [6]. Furthermore, recent studies also support the obesity paradox for all-cause mortality; a J-shaped relationship was observed between BMI and risk of HF, with the highest risk in the morbidly obese group of patients [16]. A nutritional analysis of HF patients concluded that malnutrition resulted in a significant decrease in life expectancy, while obesity was associated with a significant increase in survival [17]. In contrast, another study showed that obese subjects with HF with reduced ejection fraction (HFrEF) had higher mortality risk than lean patients [18]. Thus, there is contradictory evidence on whether the obesity paradox is observed in both HF with preserved ejection fraction (HFpEF) and HFrEF.

Several hypotheses have been put forth to explain the paradox [19]. In obese HF patients, fat may serve as a metabolic reserve and serve as source of energy [20]. Increased lipid circulation binds to endotoxins in the obese, thus improving survival. The paradox may be due to early screening of obese individuals at a young age, which could lead to early diagnosis and treatment, conferring a better survival [21]. On the other hand, some investigators propose a "lean paradox", in which HF patients with low body fat or low BMI may have poor cardiovascular outcomes [8,22]. While several of the above-referenced studies have discussed the effects of obesity on HF outcomes, intentional bariatric surgery-induced weight loss in obese patients was associated with a reduction in left ventricular hypertrophy and improvement in left ventricular diastolic function [16]. Moreover, significant weight loss induced by surgical treatment for obesity led to a reported 41% reduction in risk of HF [23]. In addition, a meta-analysis showed that intentional weight loss was associated with improvement in cardiac structure and function in obese patients [16]. Experimental studies from our group and others using animal models of HF have shown that lowering body weight in obese mice with HF improves cardiac structure and function [24,25]. While several studies support the concept of the obesity paradox in HF, some support the weight loss-induced improvement in cardiac function or survival as indicated above. The following points support our reasons for submitting in our study that an improvement in EF is a surrogate marker for survival. (1) It has been shown that higher LVEF is associated with improved survival among HFrEF patients [12–14]. (2) Furthermore, in patients with HFrEF, improved survival among the obese was associated with parallel and significant increases in LVEF [13]. (3) It has been shown that an increase in mortality rate is inversely proportional to LVEF [15]. More importantly, among patients with HFrEF, there is a linear relationship between decreasing EF and increasing mortality rates. Moreover, LVEF is an independent predictor of mortality in patients with LVEF $\leq$ 45%. Therefore, the aim of this study is to investigate whether the obesity paradox exists by analyzing LVEF in HFrEF patients in Jordan, as obesity is highly prevalent.

## 2. Methods

### 2.1. Study Design

This is a retrospective cohort study to determine the effect of BMI on EF in HFrEF patients over 12 months. Approval to conduct this study was obtained from the Jordan

University of Science and Technology and the Institutional Review Board (IRB) at the King Abdullah University Hospital (KAUH), Irbid-Jordan, on 14 January 2021 (reference code 8/137/2021). Data were retrieved from electronic hospital records from patients admitted to hospital between January 2010 and January 2020 at KAUH. Unlike the majority of studies with similar objectives, in this study we assessed the association between BMI and EF and not survival or mortality because there was no mortality over the period of analysis among the selected subjects.

### 2.2. Inclusion Criteria

Inclusion criteria were as follows: HFrEF adult (18 years and older) patients with an EF < 45% on ECHO and increased left ventricular wall thickness and having complete follow-up medical records at KAUH over 12 months.

### 2.3. Exclusion Criteria

Patients less than 18 years old, patients with type I diabetes, and patients diagnosed with cancer, autoimmune disease, immune deficiency conditions during the 12-month follow-up period were excluded from the study.

### 2.4. Statistical Analyses

Data were analyzed using the Statistical Package for Social Sciences (SPSS®25). Categorical variables were expressed as numbers and percentages, and continuous variables as means ± SD. Data normality was assessed using the Shapiro–Wilk test and the $p$-value $\geq 0.05$ indicated normally distributed data. The variables were assessed using a chi-square test or Fisher's exact test (as appropriate) for categorical data, Students' $t$-test for continuous data, and one-way ANOVA as appropriate followed by post hoc analysis. The difference between the groups was considered significant if the $p$-value was less than 0.05.

## 3. Results

### 3.1. Baseline Demographic, Clinical and Biochemical Parameters

Data from a total of 297 patients were analyzed to determine the effect of obesity on heart failure (Table 1). Overall, 65.7% ($n$ = 195) of the patients were male, while the remainder, 34.3% ($n$ = 102), were females. There was a significant difference in gender distribution within BMI categories ($p < 0.001$). A lower proportion of females was present in the lower three BMI categories compared to higher BMI groups. In addition, there was a significant difference in age between different BMI groups ($p$ = 0.015). The Obese Class III patients were significantly younger by about 10 years compared to all other BMI groups.

Several co-morbidities were present among patients selected for this study. Diabetes mellitus and hypertension were the most common and were present in 81.8% and 81.1% of patients, respectively. Other co-morbidities, such as ischemic heart disease, chronic kidney disease or dyslipidemia, were present in about one-third of patients. There was no significant difference in co-morbidities among patients of different BMI categories. In addition, there was no significant difference in systolic blood pressure (SBP) among patients of different BMI groups. However, there was a significant difference in diastolic blood pressure (DBP) among patients in different BMI categories ($p$ = 0.015). Patients in the lower three BMI categories had a lower DBP compared to those in the higher two BMI categories. Moreover, there were no significant differences in any of the lipid levels, such as total cholesterol, triglyceride, LDL, HDL or $Hb_{A1C}$, among patients in different BMI categories. Furthermore, there were no differences in markers of renal function, such as creatinine and urea. Importantly, there were no differences in EF or LVWT among patients in various BMI categories at baseline.

**Table 1.** Baseline demographics, co-morbidities and biochemical parameters.

| Variable | | All Patients | BMI Categories | | | | | p Value |
|---|---|---|---|---|---|---|---|---|
| | | | Less than 25 (*n* = 85, 28.6%) | 25–29.9 (*n* = 93, 31.3%) | 30–34.9 (*n* = 71, 23.9%) | 35–39.9 (*n* = 28, 9.4%) | ≥40 (*n* = 20, 6.7%) | |
| Gender | *Male* | 195 (65.7%) | 67 (34.4%) | 64 (68.8%) | 43 (22.1%) | 10 (5.1%) | 11 (5.6%) | <0.001 |
| | *Female* | 102 (34.3%) | 18 (17.6%) | 29 (31.2%) | 28 (27.5%) | 18 (17.6%) | 9 (8.8%) | |
| **Age: mean (SD)** | | 64.6 (12.44) | 64.8 (13.95) | 65.1 (11.71) | 65.8 (11.6) | 66.0 (11.05) | 55.4 (10.75) | **0.015** |
| Co-morbidities | *Diabetes Miletus* | 243 (81.8%) | 68 (28%) | 79 (32.5%) | 56 (23%) | 25 (10.3%) | 15 (6.2%) | 0.581 |
| | *Hypertension* | 241 (81.1%) | 72 (29.9%) | 73 (30.3%) | 59 (24.5%) | 22 (9.1%) | 15 (6.2%) | 0.753 |
| | *Ischemic Heart Disease* | 87 (29.3%) | 18 (20.7%) | 33 (37.9%) | 24 (27.6%) | 8 (9.2%) | 4 (4.6%) | 0.204 |
| | *Chronic Kidney Disease* | 83 (27.9%) | 29 (34.9%) | 26 (31.3%) | 18 (21.7%) | 7 (8.4%) | 3 (3.6%) | 0.462 |
| | *Dyslipidemia* | 58 (19.5%) | 13 (22.4%) | 16 (27.6%) | 21 (36.2%) | 5 (8.6%) | 3 (5.2%) | 0.189 |
| Lab values | *Systolic Blood Pressure* | 127.9 (22.59) | 125.0 (20.87) | 126.7 (19.68) | 128.8 (24.33) | 134.6 (24.17) | 132.4 (31.73) | 0.288 |
| | *Diastolic Blood Pressure* | 76.0 (13.56) | 74.1 (11.99) | 75.6 (11.78) | 74.9 (13.26) | 80.8 (15.39) | 82.6 (21.74) | **0.015** |
| | *Total Cholesterol* | 3.5 (1.24) | 3.6 (1.14) | 3.3 (1.06) | 3.8 (1.47) | 3.2 (1.23) | 2.7 (0.98) | 0.244 |
| | *Triglyceride* | 1.6 (1.17) | 1.5 (1.08) | 1.5 (0.89) | 1.9 (1.64) | 1.4 (0.56) | 1.6 (1.09) | 0.591 |
| | *Low Density Lipoprotein (LDL)* | 2.2 (0.94) | 2.3 (0.91) | 2.1 (0.83) | 2.3 (1.07) | 2.0 (0.99) | 1.5 (0.68) | 0.318 |
| | *High Density Lipoprotein (HDL)* | 0.87 (0.32) | 0.92 (0.32) | 0.86 (0.31) | 0.90 (0.32) | 0.84 (0.38) | 0.67 (0.23) | 0.413 |
| | HbA1c | 7.8 (2.28) | 7.6 (2.19) | 7.9 (2.38) | 8.0 (2.33) | 7.2 (1.87) | 8.9 (2.45) | 0.172 |
| | Creatinine | 186.0 (142.69) | 167.7 (142.68) | 222.5 (166.43) | 160.3 (93.93) | 183.0 (133.04) | 189.5 (157.85) | 0.648 |
| | Urea | 15.9 (10.50) | 13.9 (9.82) | 16.7 (11.28) | 16.0 (9.88) | 18.4 (9.05) | 16.6 (12.90) | 0.263 |
| | EF | 34.4 (6.13) | 33.2 (6.60) | 34.6 (5.83) | 34.8 (6.35) | 35.6 (5.54) | 35.8 (5.09) | 0.595 |
| | LVWT | 1.11 (0.07) | 1.10 (0.04) | 1.10 (0.10) | 1.10 (0.07) | 1.10 (0.05) | 1.12 (0.04) | 0.995 |

### 3.2. Changes in Cardiac Structure and Function after One Year

For all patients taken together, there was no significant change in EF after 1 year compared to baseline (Table 2). However, patients in the Obese Class I group (BMI 30.0–34.9) had a statistically significant improvement in EF, at 38.0 ± 9.81% vs. 34.8 ± 6.35% ($p = 0.004$), after 1 year. EF among patients in BMI categories lower or higher than Obese Class I showed a marginal decline, thus suggesting an inverted U-shaped relationship between BMI and EF plotted on the x- and y-axes, respectively (Figure 1). However, changes in LVWT showed a different profile. Overall, there was a small but statistically insignificant increase in LVWT among all patients ($p = 0.062$). Importantly, only patients in the healthy range of BMI less than 25 had a significantly higher LVWT after 1 year ($p = 0.045$). On the other hand, among the overweight and obese groups, no specific pattern of change in LVWT was observed after 1 year.

### 3.3. Effect of Diabetes Mellitus or Hypertension on Changes in EF

Analysis was performed to determine whether the presence or absence of diabetes had any effect on EF (Table 3). Interestingly, among non-diabetic patients, there was a significant increase in EF after 1 year. This improvement was best observed in the Obese Class I category of patients, at 7.7 ± 7.89% ($p = 0.033$). A post hoc analysis revealed that the difference in EF observed in Obese Class I patients was significantly higher than all other categories of BMI. Patients with diabetes did not have a significant increase in EF after 1 year. On the other hand, patients with hypertension had significant improvement

in EF after 1 year ($p$ = 0.020). This increase in EF was highest for the Obese Class I group of patients and was significantly higher than the healthy, overweight and Obese Class II groups of patients, as observed in the post hoc analysis. On the other hand, patients without hypertension did not see an improvement in EF.

**Table 2.** Effect of obesity on ejection fraction (EF) and left ventricular wall thickness (LVWT) after 1 year.

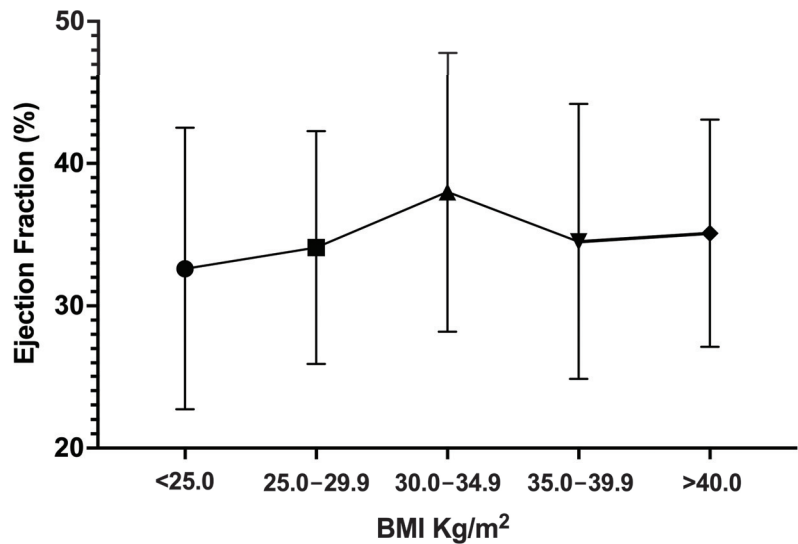| | | Ejection Fraction (Time 1) Mean (SD) | Ejection Fraction (Time 2) Mean (SD) | *p* Value | LVWT (Time 1) Mean (SD) | LVWT (Time 2) Mean (SD) | *p* Value |
|---|---|---|---|---|---|---|---|
| **All patients** | | 34.4 (6.13) | 34.7 (9.38) | 0.557 | 1.11 (0.08) | 1.12 (0.09) | 0.062 |
| **BMI groups** | **Less than 25** | 33.2 (6.60) | 32.6 (9.90) | 0.507 | 1.10 (0.04) | 1.12 (0.07) | **0.045** |
| | **25–29.9** | 34.6 (5.82) | 34.1 (8.17) | 0.463 | 1.13 (0.10) | 1.12 (0.20) | 0.200 |
| | **30–34.9** | 34.8 (6.35) | 38.0 (9.81) | **0.004** | 1.11 (0.07) | 1.12 (0.07) | 0.096 |
| | **35–39.9** | 35.6 (5.54) | 34.5 (9.67) | 0.472 | 1.10 (0.05) | 1.14 (0.06) | 0.059 |
| | **$\geq$40** | 35.8 (5.09) | 35.1 (7.97) | 0.595 | 1.12 (0.04) | 1.12 (0.05) | 1.000 |



**Figure 1.** Representation of relationship between body mass index (BMI) categories and EF.

**Table 3.** Effect of diabetes and hypertension on ejection fraction among different BMI categories.

| | | Ejection Fraction Changes Across Different BMI Categories Mean (SD) | | | | | *p* Value |
|---|---|---|---|---|---|---|---|
| | | **Less than 25** (*n* = 85, 28.6%) | **25–29.9** (*n* = 93, 31.3%) | **30–34.9** (*n* = 71, 23.9%) | **35–39.9** (*n* = 28, 9.4%) | **≥40** (*n* = 20, 6.7%) | |
| **Diabetes** | **Diabetic** (*n* = 243) | −0.4 (7.66) | −1.1 (7.37) | 2.0 (8.92) | −1.2 (8.47) | −0.9 (6.08) | 0.211 |
| | **Non-Diabetic** (*n*= 54) | −1.0 (7.83) | 2.4 (8.48) | 7.7 (7.89) | −0.3 (3.21) | 0 (2.74) | **0.033** |
| **Hypertension** | **Hypertensive** (*n* = 241) | −0.3 (7.49) | −0.2 (7.83) | 3.7 (9.20) | −1.3 (8.60) | −0.7 (6.10) | **0.020** |
| | **Non-hypertensive** (*n* = 56) | −0.2 (8.66) | −1.8 (6.76) | 0.7 (7.58) | −0.3 (6.12) | −0.6 (2.97) | 0.861 |

## 4. Discussion

The obesity paradox has been described to have a U-shaped relationship when increasing BMI is plotted on the x-axis and increasing mortality rate is plotted on the y-axis. This indicates that being overweight or mildly obese has a beneficial effect on survival. Our study investigated whether HFrEF patients in Jordan exhibit features of the obesity paradox, as characterized by a better survival or improvement in EF among obese patients compared to those with lower BMI. In our study, we identified three key findings. (1) One-year after baseline assessment, HF patients with Class I obesity had the best improvement in EF compared to those with lower or even higher BMI. (2) Class I obese HF patients without diabetes had the best improvement in EF compared to those with diabetes. (3) Hypertensive Class I obese HF patients had improvement in EF compared to those without hypertension. In summary, our study demonstrates an inverted U-shaped relationship between increasing BMI (on the x-axis) and increasing EF (on the y-axis) such that patients with mild obesity (Class I obesity) had significant improvement in EF compared to those having a lower and higher BMI (Figure 1). We therefore confirm the existence of the obesity paradox among HFrEF patients in Jordan.

Figure 1 is a representation of the average EF among patients in each BMI category. The connecting lines resemble an inverted U-shaped relationship.

Our data are consistent with a meta-analysis of individual patient data of 23,967 subjects. This study concluded that the obesity paradox was present in both HFrEF and HFpEF patients. This was characterized by a U-shaped relationship between BMI and mortality, with the lowest part of the curve showing Class I obese patients [13]. Furthermore, as part of the CHARM program, 7599 patients with HF were assessed for the obesity paradox. This study also observed that Class I obese HF patients had the highest survival rate [26]. The findings of our study, in which Class I obese patients had the maximal increase in EF after 1-year follow-up, are in close agreement with these studies. This is especially important because EF is a prognostic indicator of mortality in HF. Furthermore, a meta-analysis of nine observational studies concluded that being overweight or obese was associated with lower mortality in patients with congestive HF [12]. An inverse relationship between BMI and survival was observed. Several other studies have also reported a better survival among obese HF patients or increased mortality among low BMI patients, as reviewed in Nagarajan et al. [27].

Type 2 diabetes mellitus (T2DM) is a global epidemic; its incidence and prevalence have been on the rise over the last few decades [28]. HF is a common complication of diabetes, known as diabetic cardiomyopathy [29], and is more than twice as common among patients with diabetes compared to control subjects without diabetes [30]. Importantly, among hospitalized HFrEF patients, 42% had diabetes [31]. Thus, diabetes is significantly associated with HF. In agreement with above evidence, we observed that patients without diabetes had significant improvement in EF after 1 year. This effect was significant in the Class I obese group of patients compared to all other categories of BMI, further

supporting the obesity paradox. One previous study that examined the impact of diabetes on HFrEF among 1930 patient pairs with and without diabetes mellitus concluded that those with diabetes had a poor prognosis and experienced increased length of ICU stay or hospitalization. They also had a higher risk of events such as cardiogenic shock and death during hospitalization [32]. In our study, HFrEF patients with diabetes had poor outcomes, whereas among those without diabetes, BMI influenced EF. Similar findings have been observed in other studies, in which BMI was a significant predictor of survival among non-diabetic patients with HFrEF. However, in diabetic patients with HF, BMI was not a significant predictor of survival [33,34].

In addition, the majority of patients with diabetes (76%) in our study did not have improvement in EF. Rather, they showed a small but insignificant decline in EF. Such an insignificant effect in our study could have been due to the fact that overweight or obesity co-existed in almost 71% of individuals, and therefore, the presence of diabetes may not have had an influence on EF over and above the effect of obesity itself. Although a previous study showed that women with DM had twice the increased risk of developing HF than men [35], we did not observe an effect of gender on EF. One study examined the effect of age on the obesity paradox. This effect was more prominent with increasing age, such that older individuals had better survival compared to younger patients for a given BMI [36]. However, our study did not find an association between age and changes in EF.

In contrast to the impact of diabetes on HFrEF, we observed that Class I obese patients with hypertension had improved EF compared to those without hypertension. This is interesting, since long-standing hypertension is a risk factor for HF [37]. Moreover, the absence of hypertension is associated with a lower life-time risk of developing HF. How hypertension impacts HFrEF is worth discussing. One study has shown that among older patients with HFrEF, having an SBP < 130 mm Hg was associated with a 7% 30-day all cause mortality compared to only 4% for those with SBP $\geq$ 130 mm Hg [38]. Patients with SBP < 130 mm Hg also had a higher risk of readmission for HF at 1 year compared to those with a higher BP. Furthermore, a recent study from China that examined the effect of diabetes mellitus on HFrEF showed that there was an increase in length of hospital stay in patients without hypertension compared to those with hypertension [32]. In addition, in symptomatic patients with systolic dysfunction, having lower BP was associated with greater mortality [39]. These data suggest a poor outcome for HFrEF patients with a lower BP. Rouleau et al. observed that the lower the pre-treatment SBP, the higher the risk of death among patients with HF [40]. Another study investigated the impact of hypertension on HFpEF patients. The authors observed that SBP < 120 mm Hg was associated with a higher risk of 30-day, 12-month and 6-year all-cause mortality compared to those with SBP < 130 mm Hg [41]. In the context of HF, hypertension could simply be an indicator of force of cardiac contraction and cardiac output, since BP is a measure of the force being exerted on the arterial wall when blood is ejected out of the left ventricle [37]. Therefore, it is intriguing to hypothesize that a higher BP could simply be an indicator of better cardiac performance and higher EF.

In our study, we observed that there was no significant change in LVWT after 1 year of follow-up among all patients taken together. However, there was a significant increase in LVWT only in patients with BMI < 25 but not in other higher BMI categories. This is in contrast to several studies that have shown an increase in LVWT in obese individuals compared to lean individuals [42,43] and that LVWT is positively correlated to BMI [44].

## 5. Conclusions

Our study confirmed the existence of the obesity paradox among HFrEF patients in Jordan. This has clinical implications in that physicians and healthcare teams treating these patients can better determine management strategies for weight loss and provide information to patients regarding their prognosis. Future studies will assess the obesity paradox in HFpEF patients.

**Institutional Review Board Statement:** This study was approved on 14 January 2021, by the Institutional Review Board (IRB) at the King Abdullah University Hospital (KAUH), Irbid-Jordan (reference number 8/137/2021), and a waiver of consent was in place.

**Informed Consent Statement:** A waiver of consent was provided by the Institutional Review Board.

**Data Availability Statement:** Available upon request from corresponding author.

**Conflicts of Interest:** The authors declare no conflict of interest.

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MDPI

*Review*

# Relationship between Inflammation and Vasospastic Angina

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**Abstract:** Coronary artery spasm (CAS) is a dynamic coronary stenosis causing vasospastic angina (VSA). However, VSA is a potentially lethal medical condition with multiple presentations, including sudden cardiac death. Despite investigations to explore its pathogenesis, no single mechanism has been found to explain the entire process of VSA occurrence. The roles of elevated local and systemic inflammation have been increasingly recognized in VSA. Treatment strategies to decrease local and systemic inflammation deserve further investigation.

**Keywords:** angina; coronary artery disease; inflammation; vasospasm

## 1. History of Coronary Artery Spasm (CAS)

Prinzmetal and colleagues observed an atypical angina occurring at rest associated with an elevated ST segment on electrocardiograms transiently in patients with atherosclerotic coronary artery disease (CAD) [1]. The angina would have been due to a transient decrease in coronary blood flow, since, at rest, cardiac work is not increased. Subsequently, the term "variant angina" was suggested by Prinzmetal et al. in 1959, and they suggested that CAS was the cause because it was relieved immediately after administrating nitroglycerin. In the 1970s, variant angina was found to be caused by CAS, which was confirmed by coronary angiography. CAS can potentially occur at the site of atherosclerotic CAD [2] or diffuse spastic changes in angiographically normal coronary arteries. As a result, the investigators termed it a "variant of the variant" [3] or "vasospastic angina (VSA)" [4]. The majority of CAS cases are accompanied by ST-segment depression or T-wave changes instead of ST-segment elevation [5–7]. Therefore, the term "VSA" is a broader term to represent CAS-induced angina, irrespective of electrocardiographic manifestations. The term "variant angina" is usually expressed as CAS-induced angina associated with concurrent ST-segment elevation transiently on electrocardiogram. Recently, a Japanese guideline development by the Japanese Circulation Society has suggested that variant angina is a type of VSA [4]. The CAS experts in the Joint Working Groups in Japan [4] proposed using the term "VSA" to represent coronary vasomotor-disorder-related angina and this concept has been widely accepted [8].

## 2. How to Diagnose and Treat VSA

VSA differs from typical angina in its pathogenesis, although the exact pathophysiology of VSA is not clear at present. VSA usually occurs during resting status, especially in the night and early morning, but we found that some patients may have angina with ST-segment deviations during exercise [9]. We suggested that the spastic coronary arteries are abnormal, as the dilator response to exercise is not adequate as it would be in normal coronary arteries. There are variations in the occurrence of VSA, i.e., daily, weekly, monthly,

and circadian [10]. Many cardiologists have found that CAS can cause stable angina, acute coronary syndrome, syncope, heart failure, cardiac arrhythmias, and even sudden cardiac death [4,11]. Therefore, it is crucial to identify CAS as the underlying cause of a cardiovascular event because the treatment options will be different according to the diagnosis, i.e., pharmacological treatment first for CAS-induced and pharmacological treatment plus coronary intervention for atherosclerotic coronary artery stenoses. As a matter of fact, a correct diagnosis leads to correct treatments, and this logic of causality is the core value of clinical medicine. Recently, guidelines developed by the European Society of Cardiology for the management of survivors of sudden cardiac death have suggested that the diagnosis of CAS-induced sudden cardiac death may be considered [12]. This shows that cardiovascular events caused by CAS have been paid more and more attention.

Yasue et al. [10] found that the culprit coronary artery was patent in 17.9% of patients with acute myocardial infarction, which is similar to our report of 12% [13]. In our report, infarct-related CAS could be provoked in 95% of acute myocardial infarction patients, which suggests that a transient process of spasm and/or thrombus resolution occurs in these patients. CAS causes the formation of intracoronary thrombus [14], suggesting that CAS is a cause of acute myocardial infarction.

In the 1980s–2000s, VSA was reported to have a higher prevalence in the Japanese population compared with the western population [15,16]. Subsequently, the prevalence rates of VSA in Taiwan [17] and Korea [18] were reported to be similar to Japan [19]. The incidence of CAS provocation in our study (54%) [17] and that of Kim et al. (48%) [18] was higher than that of Bertrand et al. (12.3%) [15]. Furthermore, the rate of inducible multivessel spasms (2- and 3-vessel spasms) in our study (19%) [17] was higher than in Bertrand et al. (7.5%) [15]. However, recent European studies found that VSA is not as uncommon as previously thought in white patients with angina pectoris and myocardial ischemia with unobstructive coronary arteries [20,21]. There were no differences in patterns (more diffuse spasms and similar 2- or 3- vessel spasms) of CAS in these studies; however, higher proportions of males and smoking history were noted in the Japanese population [18]. Recent studies evaluated CAS systematically in patients who had angina or myocardial ischemia without obstructive coronary arteries. The authors found that epicardial CAS and microvascular spasm are important causes in these patients, indicating that coronary vasomotor testing should be undergone for patients with angina and no obstructive coronary arteries. The awareness of assessment for coronary vasomotor disorder has formed a consensus in the communities of cardiology and cardiovascular intervention. The probable underdiagnosis of VSA in the world, especially in the western population, should not be overlooked. In fact, the actual frequency of VSA occurrences is not easy to define because occurrences of VSA tend to fluctuate and are not necessarily symptomatic. In other words, silent ischemic CAS is a possible clinical entity. Therefore, ethnic heterogeneities in VSA require further research. No angiographic CAD can be found in one-fourth of patients with acute coronary syndrome [22,23]. CAS can be provoked in around 50% of these patients. If the electrocardiographic ST-segment changes are normalized after initial management (i.e., oxygen, aspirin, and nitroglycerin), CAS is a major factor contributing to the acute coronary syndrome. Emergency cardiac catheterization and coronary intervention is not strictly necessary under this circumstance. However, frequent attacks of VSA are a strong indication for emergency cardiac catheterization to evaluate the underlying coronary artery pathology and to perform coronary intervention if necessary. Therefore, follow-up electrocardiograms are crucial in diagnosing CAS-related acute coronary syndrome.

It is necessary to evaluate coronary function and to clarify the role of CAS in angina pectoris. Using intracoronary provocative testing to perform coronary function testing is needed and relatively safe [24], especially in patients with ischemia and nonobstructive coronary arteries [25]. Ergonovine maleate, methylergonovine maleate, and acetylcholine have been used effectively to induce CAS. The intracoronary route of ergonovine administration, with a step-wise dosing of 1, 5, 10, and 30 μg and a 3-min interval between doses, has high sensitivity and specificity in inducing CAS [26,27]. Usually, the right coronary

artery is evaluated first, then the left coronary artery. CAS is defined as a >70% reduction in the coronary arterial luminal diameter associated with chest pain and/or electrocardiographic ST-segment and T-wave changes during provocation testing [13,28]. A positive provocative test as a decrease of >90% in the coronary arterial diameter associated with chest pain and/or electrocardiographic ST-segment deviations during the provocation testing has been suggested by a Japanese guideline [4]. However, Yasue et al. [29] suggested that myocardial ischemia could be caused by reduced coronary blood flow for long enough. Therefore, a definition of coronary artery lumen reduction seems to be of no absolute necessity. The core of a positive CAS provocation test result is concurrent angina and/or ischemic electrocardiographic changes during testing. Therefore, simultaneous patient symptom inquiry and electrocardiographic monitoring are absolutely necessary. Pre-testing, liquid nitroglycerin must be well prepared, and 50–600 µg of intracoronary nitroglycerin is administered once a CAS has been diagnosed. Certainly, intracoronary ergonovine administration must be stopped before intracoronary nitroglycerin administration. Only methylergonovine maleate is available in Taiwan; therefore, it was used in our prior studies with the intracoronary dose protocol the same as for ergonovine maleate. This procedure was safe with low complication rates [24,30]. Reported complications of intracoronary provocative testing for CAS include angina, atrial or ventricular arrhythmias, hypotension, nausea, vomiting, and flushing. There have been no reports regarding procedure-related mortality or myocardial infarction. It is recommended that CAS provocative testing should be undergone in a cardiac catheterization because of possible fatal or non-fatal arrhythmias occurring during testing. Therefore, it is not advisable not to undergo intracoronary provocative testing for fear of complications, as with all cardiac interventions. A complete and correct diagnosis should be made for the patient who has angina and no obstructive coronary arteries as long as there is detailed and complete preparation before testing. Theoretically, the diagnosis of VSA should be made according to the intracoronary provocation testing result; however, it is not practical to undertake intracoronary provocation testing immediately after an attack of VSA in every patient. However, some clues are more likely to reflect VSA: (1) chest pain occurs in resting status, especially at night and in the early morning; (2) chest pain is associated with concurrent electrocardiographic ST-segment and T-wave changes; (3) chest pain is quickly relieved by nitroglycerin in any form. Even so, it is still advisable to undertake intracoronary provocative testing if there is no contraindication.

In addition to intracoronary stress testing, other non-invasive stress modalities have been used to diagnose VSA, such as hyperventilation [31] and stress echocardiography using either cold-pressor testing or intravenous ergonovine testing [32,33]. In 1999, Nakao et al. [31] studied 206 angiographically confirmed CAS patients (spasm group) and 183 non-angina and non-angiographically-inducible CAS patients (non-spasm group) using vigorous hyperventilation for 6 min in the early morning. Of these 206 patients, 127 had positive electrocardiographic responses to the test; however, all negative responses were noted in the non-spasm group. As a result, the sensitivity and specificity of hyperventilation testing for diagnosing CAS were 62% and 100%, respectively. The postulated mechanism is that respiratory alkalosis induced by hyperventilation enhances Na-H exchange followed by Na-Ca exchange, subsequently causing increased intracellular calcium concentration. In 2001, Hirano et al. [32] reported 2-dimensional echocardiographic stress testing to evaluate CAS. The stress testing includes hyperventilation for 6 min, followed by cold water pressor stress for 2 min. The whole process was closely monitored by continuous electrocardiograms and echocardiograms. The sensitivity, specificity, and diagnostic accuracy of this stress testing protocol for detecting CAS were 48%, 100%, and 60%, respectively. These results mean that these tests are specific for CAS. In other words, CAS truly exists when angina occurs after hyperventilation. In 2005, Song et al. [33] reported the role of intravenous ergonovine stress echocardiography in the diagnosis of CAS. The positive rate was 8.6% for detecting CAS, and no procedure-related mortality or myocardial infarction was noted. Based on the above studies, it is suggested that these non-invasive modalities are al-

ternative methods in diagnosing VSA if there are contraindications to undergoing invasive coronary angiography, and these are suggested to be performed by experienced physicians. Although diagnosis of VSA can be made invasively and non-invasively, contraindications to performing these tests still exist and need attention. Absolute contraindications to undergoing CAS provocation testing include severe left ventricular dysfunction, moderate to severe aortic stenosis, high-grade left main coronary artery stenosis, severe hypertension (systolic blood pressure > 180 mmHg), and pregnancy [34]. Relative contraindications include significant coronary artery disease, recent myocardial infarction, uncontrolled or unstable angina, and uncontrolled ventricular arrhythmia. Based on the above literature reviews, taking a thorough medical history and a follow-up series of electrocardiographic ST-segment and T-wave changes are the bases for diagnosing VSA.

Calcium antagonists are the first-line therapy in the treatment of VSA [4,10,35]. Calcium antagonists are suggested to be given before bedtime at night because VSA frequently occurs between midnight and early morning. Furthermore, the doses of calcium antagonists for VSA are not the same as those for treating hypertension; a larger dose of calcium antagonist is usually needed, e.g., diltiazem 240–360 mg/day. Controlling VSA may occasionally require two distinct chemical classes of calcium antagonists, i.e., dihydropyridine and non-dihydropyridine. In contrast, a non-selective β-blocker, propranolol, may aggravate VSA [36]. Nitrate can relieve CAS promptly, but its role in VSA prevention is limited by tolerance and poor long-term clinical outcomes [37]. Some clinical research shows that magnesium [38], antioxidants [39,40], and Rho-kinase inhibitors [41] are also helpful for treatment of VSA. Additionally, precipitating factors for VSA should be absolutely avoided, e.g., alcohol, cigarette smoking, and propranolol [11]. Coronary intervention is not helpful for drug-refractory VSA [42] and is contraindicated in patients without angiographical CAD because of the presence of diffuse spastic characteristics in the setting of CAS [4]. Cardioverter defibrillator implantation with adequate pharmacological therapy for CAS was suggested to be an appropriate option for patients who had syncope or ventricular tachycardia or had survived hospital cardiac arrest [43]. Pharmacological treatment for VSA with calcium antagonists is suggested to be lifelong, not only because of persistent long-term spasticity of the coronary arteries [44] but also the probability of silent myocardial ischemia. Silent myocardial ischemia caused by any pathologies could be complicated by fatal or nonfatal cardiovascular events, even, as previously mentioned, ventricular arrhythmias and cardiac death. Despite the 5.5–11% recurrence rate of VSA, the long-term prognosis of VSA is good if adequate treatment is prescribed [17,45].

Summary: VSA must be diagnosed and treated correctly based on the following [46]:

1. Angina occurs at rest and is promptly relieved by administering nitrates, but the diagnosis of VSA must further be correctly confirmed;
2. VSA can present as stable angina, acute coronary syndrome, syncope, cardiac arrhythmias, heart failure, and sudden death;
3. Without intracoronary testing for vasomotion, angiographically patent coronary arteries should not be interpreted as normal coronary arteries;
4. Intracoronary provocative testing must be well prepared and undertaken based on the guidelines and should not be considered a risky procedure;
5. Calcium antagonists are the first choice for the treatment of VSA and should be given at the right time and in the right doses.

### 3. Relation of Local and Systemic Inflammation to VSA

No single mechanism can be held responsible for the development of CAS. Some mechanisms have been proven to play a role in CAS causing VSA, i.e., allergy [47], oxidative stress [48], endothelial dysfunction [49], deficient aldehyde dehydrogenase 2 activities [50], chronic low-grade inflammation [51], magnesium deficiency [38], and hypercontraction of coronary artery smooth muscle [52]. Furthermore, age, cigarette smoking, and high-sensitivity C-reactive protein (hs-CRP) are risk factors for VSA [53]. Other factors act as inducers for VSA occurrence [11], such as physical and/or mental stress, alcohol consump-

tion, Valsalva maneuver, hyperventilation, and other pharmacological agents, such as propranolol, ergot alkaloids, sympathomimetics and parasympathomimetics, and cocaine. Chronic low-grade inflammatory conditions seem to play the central role, interacting with each of the above-mentioned mechanisms. Although different pathophysiologies exist in VSA, the final pathway is contraction of coronary artery smooth muscle, clinically causing VSA [54]. Based on the above prior studies, it is suggested that the underlying mechanism in the development of CAS is multifactorial. The etiology of the hyperreactivity of the coronary vessels is unclear but could be related to endothelial dysfunction and the primary smooth muscle cells of the coronary vessels, which might have impaired regulatory mechanisms for vasoconstriction and vasodilation. Balances within the sympathetic and parasympathetic tone also regulate the coronaries' flow. Since multiple factors can contribute to the development of VSA, an occurrence of VSA is variable and therefore unpredictable [44].

In 1978, Lewis and colleagues [55] described a patient who was deceased due to cardiogenic shock because of inferior wall ST-segment elevation associated with localized pericarditis. These investigators initially suggested an interaction between chronic inflammation and CAS. Subsequently, Forman et al. [56] found a VSA patient who presented with sudden death, in whom infiltrating mast cells were found at the adventitia of a spastic coronary artery. In 1988, Ferguson et al. [57] reported a 17-year-old boy who had developed two episodes of VSA following assumed acute viral myocarditis. In 1991, Iwasaki et al. [58] reported CAS in a 59-year-old male with biopsy-proven acute myocarditis. In 2008, Yilmaz et al. [59] found that CAS without CAD occurs in 70% of endomyocardial biopsy-proven PVB19 myocarditis and suggested that CAS plays an important role in the occurrence of angina pectoris in these patients. Other studies have also found intimal injury and neointimal hyperplasia with infiltrating inflammatory cells in coronary plaques or arteries in patients with VSA [60,61]. Despite a lack of angiographical evidence of coronary artery narrowing, diffuse intimal thickening in spastic arteries has been demonstrated by intracoronary ultrasound [62]. Using $^{18}$F-fluorodeoxyglucose positron emission tomography/computed tomography, inflammatory changes in coronary adventitia and perivascular adipose tissue were found to be associated with CAS in VSA patients [63]. Coronary perivascular $^{18}$F-fluorodeoxyglucose uptake decreased after prescription of a calcium antagonist in patients with VSA. Furthermore, adventitial vasa vasorum significantly increased in VSA patients, as confirmed by optical coherence tomography analysis. All the above findings suggest that local coronary inflammatory changes play a role in the early anatomical changes in the coronary arteries in CAS (Table 1), which was also suggested by Marzilli and colleagues [64]. These early anatomical changes in the coronary arteries in CAS might induce subsequent functional changes in these arteries, which might be the basis of future characteristics of spastic coronary arteries.

**Table 1.** Relationship between inflammation and vasospastic angina.

| Inflammatory Status | Comments |
| --- | --- |
| Local | |
| Pericarditis | Case report |
| Mast cells infiltration in adventitia of spastic coronary artery | Case report |
| Acute viral myocarditis | Biopsy-proven |
| Intimal injury and intimal hyperplasia | Histology |
| Diffuse intimal thickening | Intravascular ultrasound |
| Inflammation of Coronary adventitia and perivascular adipose tissue | Positron emission tomography |
| Adventitial vasa vasorum increase | Optical coherence tomography |

**Table 1.** *Cont.*

| Inflammatory Status | Comments |
|---|---|
| Systemic | |
| Elevated circulatory inflammatory and adhesion markers | Plasma/serum studies |
| Elevated peripheral leukocyte ROCK activity | Protein expression studies |
| Kounis syndrome | Clinical disease entity association |
| Asthma association | Clinical disease entity association |
| Anxiety/depression association | Clinical disease entity association |
| Insulin resistance | Clinical disease entity association |
| Cigarette smoking association | Clinical risk factor |

Increased levels of soluble intercellular adhesion molecule-1 or secretory type II phospholipase A2 have been noted in patients with VSA [65,66]. Our prior serum inflammatory biomarker studies also found increased levels of hs-CRP, interleukin-6, monocyte chemoattractant protein-1, soluble intercellular adhesion molecule-1, and soluble vascular adhesion molecule-1 in patients with VSA [51,67], indicating that systemic inflammatory changes associated with subsequent endothelial dysfunction are present in spastic coronary arteries. Endothelial dysfunction is the earliest process of atherosclerotic lesion formation [68]. Furthermore, atherosclerosis impairs the coronary arterial vasodilator function, which is an important function of endothelium [69]. Recently, we also found elevated peripheral leukocyte Rho-associated coiled-coil-containing protein kinase activity in patients with VSA [70]. Rho-associated coiled-coil-containing protein kinase activity was decreased in the VSA group after treatment with antispastic agents for 3 months. Rho-associated coiled-coil-containing protein kinase activity was independently associated with diagnosis of VSA and was found to be correlated with VSA activity. Rho-associated coiled-coil-containing protein kinase activation has been noted in association with attenuated endothelial nitric oxide synthase expression [71], increased vascular smooth muscle cell DNA synthesis and migration [72], and increased monocyte adhesion and spreading [73]. Some molecular studies in the porcine model with interleukin-1 beta showed that the expressions of Rho-kinase mRNA and RhoA mRNA were increased in the spastic coronary segment as compared with the control coronary segment [74]. Using a Rho-kinase inhibitor, Y-27632, not only inhibited serotonin-induced vascular smooth muscle hypercontraction but also accentuated myosin binding subunit phosphorylation [75]. The above molecular studies indicate that Rho-kinase is upregulated at the spastic site and causes vascular smooth muscle hypercontraction. Therefore, the pathogenesis of VSA could be a combination and interplay of endothelial dysfunction, systemic inflammation, and smooth muscle hypercontraction. Our series of CAS studies and other prior CAS studies do not include patients with obstructive CAD; low-grade systemic inflammation is present in these VSA patients, similar to that found in obstructive CAD patients. Therefore, it is reasonable to infer that coronary arteries undergoing CAS are not normal, and that systemic inflammatory status exists in VSA, because abnormal endothelial function and diffuse intimal thickening causing inadequate nitric oxide synthesis is observed in these patients.

In 1991, Kounis et al. [47] postulated a concept of allergic angina based on observing an acute allergic condition associated with acute coronary syndromes. They then suggested that histamine, the main amine during allergy, could induce CAS manifested as VSA or acute myocardial infarction. Subsequently, they modified their understanding of the Kounis syndrome towards mast cell activation [76], further making an argument for allergic inflammatory-response-induced CAS.1 There are three variants of the Kounis syndrome [76], i.e., Type I: allergic VSA due to endothelial dysfunction in patients without underlying CAD, Type II: an allergic reaction causing CAS or plaque erosion in patients with underlying asymptomatic CAD, and Type III: an allergic CAS in the setting of coronary thrombosis, including stent thrombosis. Our prior case report demonstrated that type I Kounis syndrome occurred in a 45-year-old sigmoid cancer patient who had drug-allergic

VSA with the chemotherapy agent oxaliplatin [77]. Because treatment strategies for Kounis syndrome and asthma are not exactly the same as for pure VSA, knowledge of individual hypersensitivity is required.

Using the National Health Insurance Research Database, we also noticed that asthma is independently associated with new-onset VSA (odds ration = 1.85) [78], providing further evidence of the interplay between allergic reaction and CAS. In this study, the risk of new-onset VSA was higher in prior steroid users irrespective of the oral (odds ratio = 1.22) or inhaled route (odds ratio = 1.89). Further analysis showed that the prevalence of asthma in VSA patients (4.4%) was the highest, followed by patients who had VSA associated with atherosclerotic coronary artery disease (2.6%) and atherosclerotic coronary artery disease treated by coronary intervention (1.8%). These results further indicate that an interplay exists between the bronchial spasm of asthma and the CAS of VSA. Inflammation can contribute to the occurrence of asthma [79]. As a result, the inflammatory process plays an important role in the occurrence of bronchial spasm and CAS.

Smoking is an important association factor for VSA [80]. Our investigation [81] reported an odds ratio of 2.58, similar to a prior CAS investigation's 2.41 [76]. A synergistic interaction between smoking and hs-CRP was further identified in the study [81]. Among smokers, the interaction was linear and monotonic. In non-smokers, a threshold effect of hs-CRP was observed on VSA. After adjusting for hs-CRP as a confounder in analyzing the impact of smoking on VSA development, a decreased odds ratio was found, suggesting hs-CRP as an important covariate of VSA. Furthermore, we found that the relation of hs-CRP to VSA is different between genders [53]. A non-threshold model for male patients and a threshold model for female patients can be interpreted as more male smokers (lifestyle) and older smokers (induction time) contributing to the natural history of VSA development. Interestingly, hypertension was found to be negatively associated with VSA [82], suggesting that VSA is different from coronary atherosclerosis in terms of pathogenesis. Recently, our cellular study [83] also noted that elevated levels of monocytic interleukin-6 and $\alpha7$ nicotinic acetylcholine receptor mRNA expression and protein production are related to the interaction between nicotine and C-reactive protein. This effect is positive on the occurrence of CAS. Another big data analysis using the National Health Insurance Research Database found that anxiety and depression diagnosis are risk factors for VSA [84]. Patients with anxiety and depression have a higher risk of new-onset CAS compared with new-onset atherosclerotic coronary artery disease (odds ratios = 2.29 and 1.34, respectively). Further analysis found that a stronger risk association is noted when comparing CAS with a control group without atherosclerotic coronary artery disease or CAS (odds ratios = 5.20 and 1.98, respectively). In this study, there was no gender difference in the association of anxiety and depression with CAS. An elevated inflammatory condition in patients with depression and anxiety with potential causality has been documented in United Kingdom Biobank and Netherlands Study of Depression and Anxiety cohorts [85]. Using the National Health Insurance Research Database, we noted that CAS is associated with incident diabetes irrespective of gender, indicating a link between the inflammation of VSA and the insulin resistance of incident diabetes [86]. Insulin resistance is a central marker of metabolic syndrome, and its positive association with VSA has been identified [87,88]. Inflammation exists in the state of insulin resistance [89]. Insulin resistance is associated with compensatory hyperinsulinemia, which further causes endothelial dysfunction [90]. However, VSA does not occur in every patient with endothelial dysfunction [25]. A pathological phenomenon does not necessarily lead to clinical disease. Therefore, an association between systemic inflammation and VSA (Table 1) is further suggested [91–93].

## 4. Conclusions

VSA is a potentially lethal medical condition with multiple presentations. A detailed medical history and a follow-up series of electrocardiographic ST-segment and T-wave changes are the bases for diagnosing VSA. To identify the underlying cause of angina, especially in patients with no obstructive coronary arteries, is crucial in the primary coronary

interventional era. Only correct diagnosis can lead to correct treatment. With the advancement of medical diagnostic imaging capabilities, discovery of the pathogenesis of CAS has become possible. Local and systemic inflammation in association with VSA is increasingly being recognized. Therefore, effective treatment strategies to decrease inflammation are worthy of further investigation.

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*Article*

# Optimal Heart Rate Control Improves Long-Term Prognosis of Decompensated Heart Failure with Reduced Ejection Fraction

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**Abstract:** *Background and Objectives:* An elevated heart rate is an independent risk factor for cardiovascular disease; however, the relationship between heart rate control and the long-term outcomes of patients with heart failure with reduced ejection fraction (HFrEF) remains unclear. This study explored the long-term prognostic importance of heart rate control in patients hospitalized with HFrEF. *Materials and Methods*: We retrieved the records of patients admitted for decompensated heart failure with a left ventricular ejection fraction (LVEF) of ≤40%, from 1 January 2005 to 31 December 2019. The primary outcome was a composite of cardiovascular death or hospitalization for heart failure (HHF) during follow-up. We analyzed the outcomes using Cox proportional hazard ratios calculated using the patients' heart rates, as measured at baseline and approximately 3 months later. The mean follow-up duration was 49.0 ± 38.1 months. *Results*: We identified 5236 eligible patients, and divided them into five groups on the basis of changes in their heart rates. The mean LVEFs of the groups ranged from 29.1% to 30.6%. After adjustment for all covariates, the results demonstrated that lesser heart rate reductions at the 3-month screening period were associated with long-term cardiovascular death, HHF, and all-cause mortality (*p* for linear trend = 0.033, 0.042, and 0.003, respectively). The restricted cubic spline model revealed a linear relationship between reduction in heart rate and risk of outcomes (*p* for nonlinearity > 0.2). *Conclusions*: Greater reductions in heart rate were associated with a lower risk of long-term cardiovascular death, HHF, and all-cause mortality among patients discharged after hospitalization for decompensated HFrEF.

**Keywords:** heart rate; heart failure; mortality

## 1. Introduction

A high resting heart rate is an independent risk factor for all-cause mortality, cardiovascular mortality, and cardiovascular events among the general population [1,2] as well as among patients with cardiovascular disease, coronary artery disease, hypertension, heart failure, and diabetes [3–9]. The relationship between heart rate and adverse outcomes may

be mediated by the effects of heart rate on coronary blood flow, cardiac contractility, and energy expenditure [7,10]. Reducing a patient's heart rate can reduce afterload, relieve left ventricular wall stress, and increase the stroke volume of the left ventricle, thus improving the patient's heart function and alleviating their cardiovascular symptoms [11]. These findings suggest that physicians should implement interventions to reduce the heart rates of patients with HFrEF and improve their clinical outcomes.

Numerous studies have explored the effects of heart rate control on patients with heart failure. A randomized controlled trial involving patients with HFrEF, the Ivabradine and Outcomes in Chronic Heart Failure (SHIFT) study, demonstrated that reductions in heart rate due to ivabradine benefit patients with HFrEF who have heart rates of >70 bpm, despite receiving guideline-directed therapies, including beta blockers [12]. The rates of major adverse cardiovascular events, namely hospitalization for heart failure (HHF) and cardiovascular death, were significantly lower in the ivabradine group than in the placebo group, especially among the patients with higher baseline heart rates.

The importance of heart rate monitor and control have been addressed in major guidelines [13,14]; however, the relationship between heart rate reductions and health outcomes have not been thoroughly evaluated. In addition, few studies have analyzed the long-term outcomes of heart rate control for patients discharged after hospitalization for decompensated HFrEF. We conducted this study to evaluate the effect of heart rate reductions on the long-term outcomes of patients with HFrEF discharged from the hospital through an analysis of records from multiple healthcare institutions.

## 2. Method

### 2.1. Data Source

This study was conducted using the Chang Gung Research Database (CGRD), a de-identified database managed by the largest healthcare provider in Taiwan, the Chang Gung Memorial Hospital (CGMH) healthcare system. The CGMH system is multi-institutional, comprising seven healthcare institutions (four tertiary academic medical centers and three teaching hospitals) across Taiwan. The use of data from the CGRD as the basis for accurate estimates in medical studies has been validated [15]. The Chang Gung Memorial Hospital Institutional Review Board approved this study and waived the requirement for informed consent. The patients' records were anonymized and de-identified before analysis. For data generated before 2015, we used the *International Classification of Diseases*, *Ninth Revision*, *Clinical Modification* (*ICD-9-CM*) for diagnosis, whereas for data generated after 2016, we used both the *ICD-9-CM* and the *ICD Tenth Revision* (*ICD-10-CM*). More information regarding the CGRD has been published in other articles [15,16]. This study was conducted in accordance with the principles outlined in the Declaration of Helsinki [17].

### 2.2. Study Group and Cohort

From the CGRD, we retrieved the records of patients admitted for decompensated heart failure with a left ventricular ejection fraction (LVEF) of ≤40%, from 1 January 2005 to 31 December 2019. The index date was the date when each patient was discharged after index heart failure admission. Each patient's LVEF was determined on the basis of the echocardiography report generated during the index admission. Each patient's baseline heart rate was defined as their first heart rate recorded after the index admission. The first recorded heart rate at admission is the condition before medications or treatments for heart failure control. Each patient's follow-up heart rate was defined as their heart rate recorded at the 3-month screening period in the outpatient department. Clinically, physicians may frequently adjust the medication and treatment for a short period after discharge. It was noted that the medications prescribed for heart failure were less changed until a period of 2–4 months after discharge. Thus, we chose the 3 months after discharge as the screening period. Patients were excluded if they were aged younger than 20 years, had a baseline heart rate of <70 bpm, had a diagnosis of atrial fibrillation or atrial flutter before or during the index admission, or did not survive to discharge. Patients who died, presented with

heart failure exacerbation and required readmission before the 3-month screening period, had follow-up periods of <90 days, or lacked follow-up heart rate measurements, were also excluded (Figure 1). A total of 5236 patients with decompensated heart failure and an LVEF of ≤40% requiring hospitalization with follow-up durations of over 3 months were determined to be eligible for inclusion.
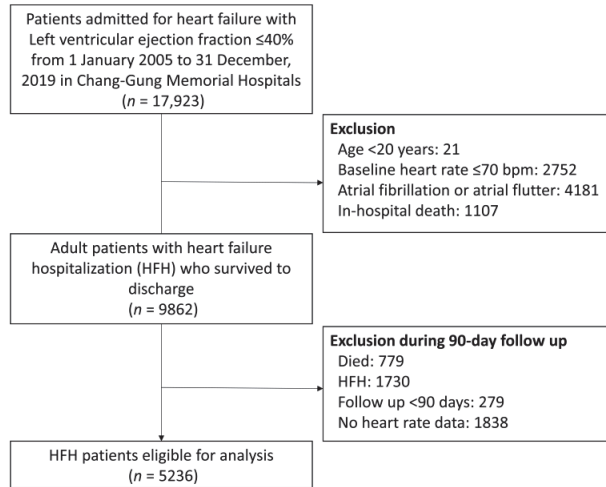


**Figure 1.** Flowchart of patient inclusion and exclusion.

*2.3. Covariate Measurements*

The covariates of interest were demographic characteristics (age, sex, smoking status, and body mass index), baseline vital signs (systolic and diastolic blood pressure and heart rate), number of HHFs in the previous year, number of HHFs in the previous 3 years, comorbidities (coronary artery disease, myocardial infarction, hypertension, dyslipidemia, diabetes mellitus, chronic kidney disease, dialysis, stroke, chronic obstructive pulmonary disease, peripheral arterial disease, and liver cirrhosis), medications used during the index admission (angiotensin-converting enzyme inhibitors [ACEIs] or angiotensin receptor blockers [ARBs], beta blockers, and 11 others), laboratory test results (serum creatinine levels and 15 others), echocardiography results, in-hospital events, and heart failure medications taken within 3 months of discharge (Tables 1 and 2). The echocardiographic parameters of interest were the LVEF, left ventricular end-diastolic diameter, left ventricular end-systolic diameter, left atrium diameter, and mitral regurgitation severity. The in-hospital covariates during the index admission were hospital stay (in days), intensive care unit (ICU) stay (in days), episodes of shock (use of inotropic agents, intra-aortic balloon pumps, or extracorporeal membrane oxygenation), intubation, episodes of acute coronary syndrome, and percutaneous coronary interventions. The heart failure medications of interest were beta blockers, ivabradine, digoxin, ACEIs/ARBs, angiotensin receptor–neprilysin inhibitors (ARNIs), mineralocorticoid receptor antagonists (MRAs), and loop diuretics.

*2.4. Outcome Definitions*

The primary outcome was a composite of cardiovascular death or HHF during follow-up. The secondary outcomes were cardiovascular death, HHF, and all-cause mortality. HHF was defined as unscheduled hospitalization during which the patient required at least one treatment, which may have included diuretics, nitrites, or inotropic agents. The patients' dates, places, and causes of death were linked to the Taiwan Death Registry database. The definition of cardiovascular death encompassed death due to acute myocardial infarction; sudden cardiac death; and death due to heart failure, stroke, cardiovascular procedures, cardiovascular hemorrhage, or other cardiovascular causes [18]. The follow-up period

was defined as the period from the date of the index hospitalization to the date of death, outcome occurrence, or loss to follow-up or 31 December 2020, whichever occurred first.

**Table 1.** Baseline characteristics, comorbidities, laboratory data, and echocardiography results of patients grouped by changes in heart rate from the admission day to the 90th day after discharge.

| Variable | n | Decrease ≥30 (n = 798) | Decrease 20–29 (n = 744) | Decrease 10–19 (n = 1058) | Decrease <10 (n = 1188) | Increase 1–10 (n = 849) | Increase >10 (n = 599) | p Trend |
|---|---|---|---|---|---|---|---|---|
| Age, year | 5236 | 62.9 ± 16.7 | 63.6 ± 15.7 | 63.5 ± 15.2 | 63.2 ± 14.9 | 63.1 ± 15.1 | 61.0 ± 15.4 | 0.020 |
| Male | 5236 | 553 (69.3) | 521 (70.0) | 720 (68.1) | 804 (67.7) | 594 (70.0) | 442 (73.8) | 0.215 |
| Smoking | 5236 | 315 (39.5) | 269 (36.2) | 381 (36.0) | 428 (36.0) | 310 (36.5) | 238 (39.7) | 0.919 |
| BMI, kg/m$^2$ | 4920 | 24.9 ± 5.1 | 24.8 ± 4.6 | 25.0 ± 4.8 | 25.1 ± 4.7 | 25.1 ± 5.1 | 25.2 ± 5.1 | 0.092 |
| Baseline vital sign | | | | | | | | |
| SBP, mmHg | 5236 | 134.9 ± 28.2 | 134.9 ± 27.2 | 134.6 ± 27.0 | 132.9 ± 24.8 | 129.3 ± 24.6 | 129.0 ± 23.2 | <0.001 |
| DBP, mmHg | 5235 | 84.0 ± 20.1 | 81.7 ± 17.6 | 80.7 ± 18.4 | 78.7 ± 16.0 | 76.9 ± 16.0 | 77.9 ± 15.4 | <0.001 |
| Heart rate, beat/minute | 5236 | 113.9 ± 18.0 | 98.3 ± 11.2 | 92.3 ± 12.1 | 87.0 ± 10.9 | 84.3 ± 10.2 | 81.6 ± 9.2 | <0.001 |
| HF admission in the previous year | 5236 | 93 (11.7) | 112 (15.1) | 159 (15.0) | 196 (16.5) | 148 (17.4) | 123 (20.5) | <0.001 |
| No. of HF admission in the previous 3 years | 5236 | | | | | | | <0.001 |
| 0 | | 687 (86.1) | 613 (82.4) | 866 (81.9) | 933 (78.5) | 659 (77.6) | 444 (74.1) | |
| 1 | | 89 (11.2) | 106 (14.2) | 156 (14.7) | 200 (16.8) | 144 (17.0) | 122 (20.4) | |
| ≥2 | | 22 (2.8) | 25 (3.4) | 36 (3.4) | 55 (4.6) | 46 (5.4) | 33 (5.5) | |
| Comorbidity | | | | | | | | |
| Coronary artery disease | 5236 | 414 (51.9) | 408 (54.8) | 621 (58.7) | 727 (61.2) | 482 (56.8) | 348 (58.1) | 0.005 |
| Myocardial infarction | 5236 | 62 (7.8) | 82 (11.0) | 118 (11.2) | 166 (14.0) | 109 (12.8) | 71 (11.9) | 0.001 |
| Hypertension | 5236 | 506 (63.4) | 499 (67.1) | 732 (69.2) | 817 (68.8) | 584 (68.8) | 393 (65.6) | 0.170 |
| Dyslipidemia | 5236 | 299 (37.5) | 310 (41.7) | 443 (41.9) | 539 (45.4) | 385 (45.3) | 239 (39.9) | 0.024 |
| Diabetes mellitus | 5236 | 318 (39.8) | 360 (48.4) | 529 (50.0) | 590 (49.7) | 430 (50.6) | 298 (49.7) | <0.001 |
| Chronic kidney disease | 5236 | 313 (39.2) | 316 (42.5) | 416 (39.3) | 495 (41.7) | 313 (36.9) | 227 (37.9) | 0.202 |
| Dialysis | 5236 | 48 (6.0) | 76 (10.2) | 109 (10.3) | 118 (9.9) | 88 (10.4) | 61 (10.2) | 0.015 |
| Stroke | 5236 | 52 (6.5) | 62 (8.3) | 79 (7.5) | 103 (8.7) | 62 (7.3) | 46 (7.7) | 0.547 |
| Chronic obstructive Pulmonary disease | 5236 | 139 (17.4) | 142 (19.1) | 160 (15.1) | 205 (17.3) | 167 (19.7) | 108 (18.0) | 0.475 |
| Peripheral arterial disease | 5236 | 55 (6.9) | 63 (8.5) | 103 (9.7) | 101 (8.5) | 87 (10.2) | 68 (11.4) | 0.005 |
| Liver cirrhosis | 5236 | 24 (3.0) | 19 (2.6) | 32 (3.0) | 29 (2.4) | 38 (4.5) | 26 (4.3) | 0.041 |
| Laboratory data | | | | | | | | |
| BNP, pg/mL | 2884 | 1230 (599, 2239) | 1168 (565, 2290) | 1240 (612, 2449) | 1100 (514, 2190) | 1155 (509, 2580) | 1166 (500, 2239) | 0.382 |
| BUN, mg/dL | 5014 | 29.6 ± 20.9 | 31.2 ± 21.9 | 30.8 ± 22.8 | 31.0 ± 23.5 | 30.2 ± 21.7 | 30.1 ± 22.9 | 0.943 |
| Creatinine, mg/dL | 5219 | 2.0 ± 2.1 | 2.2 ± 2.4 | 2.2 ± 2.5 | 2.2 ± 2.7 | 2.2 ± 2.5 | 2.2 ± 2.6 | 0.364 |
| eGFR, mL/min/1.73 m$^2$ | 5219 | 58.0 ± 32.7 | 57.9 ± 35.1 | 59.3 ± 34.9 | 60.7 ± 36.4 | 61.8 ± 36.4 | 63.3 ± 36.4 | <0.001 |
| Sodium (Na), mEq/L | 5197 | 137.8 ± 4.6 | 137.6 ± 4.5 | 137.9 ± 4.4 | 137.9 ± 4.3 | 138.1 ± 4.0 | 138.4 ± 3.9 | 0.001 |
| Potassium (K), mEq/L | 5204 | 3.9 ± 0.7 | 4.0 ± 0.7 | 4.0 ± 0.6 | 4.0 ± 0.6 | 4.0 ± 0.6 | 4.0 ± 0.6 | 0.045 |
| Uric acid, mg/dL | 3275 | 8.0 ± 2.8 | 7.8 ± 2.5 | 7.7 ± 2.4 | 7.5 ± 2.5 | 7.7 ± 2.5 | 7.7 ± 2.8 | 0.028 |
| AST, U/L | 4014 | 34 (24, 62) | 31 (22, 49) | 29 (22, 45) | 30 (22, 46) | 28 (21, 42) | 29 (21, 44) | <0.001 |
| ALT, U/L | 4833 | 27 (17, 54) | 25 (16, 45) | 24 (15, 42) | 25 (16, 44) | 24 (16, 40) | 22 (14, 38) | <0.001 |
| LDL-C, mg/dL | 4131 | 82.9 ± 47.8 | 83.8 ± 48.1 | 85.7 ± 49.7 | 86.6 ± 50.1 | 86.0 ± 49.2 | 88.1 ± 48.8 | 0.056 |
| Total cholesterol, mg/dL | 4275 | 167.7 ± 44.5 | 168.6 ± 46.8 | 167.8 ± 45.6 | 170.3 ± 45.3 | 166.6 ± 45.9 | 167.8 ± 44.9 | 0.859 |
| Hemoglobin, g/dL | 5228 | 12.7 ± 2.6 | 12.5 ± 2.6 | 12.5 ± 2.5 | 12.4 ± 2.5 | 12.4 ± 2.5 | 12.6 ± 2.4 | 0.613 |
| Total bilirubin, mg/dL | 3315 | 1.1 ± 0.9 | 1.0 ± 0.8 | 0.9 ± 0.7 | 0.9 ± 0.7 | 0.9 ± 0.7 | 0.9 ± 0.6 | <0.001 |
| Albumin, mg/dL | 3734 | 3.5 ± 0.6 | 3.5 ± 0.6 | 3.5 ± 0.6 | 3.5 ± 0.6 | 3.5 ± 0.6 | 3.5 ± 0.5 | 0.023 |
| Platelet, count × 10$^3$ | 5225 | 233.2 ± 86.1 | 224.3 ± 84.3 | 227.6 ± 82.4 | 218.2 ± 75.1 | 217.5 ± 82.8 | 220.9 ± 76.5 | <0.001 |
| WBC, count × 10$^3$ | 5228 | 10.8 ± 4.7 | 9.3 ± 3.8 | 9.2 ± 3.8 | 8.6 ± 3.5 | 8.5 ± 3.5 | 8.5 ± 3.2 | <0.001 |
| Echocardiography result | | | | | | | | |
| LVEF, % | 5236 | 29.1 ± 7.7 | 29.8 ± 7.5 | 30.2 ± 7.3 | 30.5 ± 7.0 | 30.5 ± 6.9 | 30.6 ± 7.0 | <0.001 |
| LVEDD, mm | 5233 | 58.7 ± 9.1 | 59.1 ± 8.6 | 59.4 ± 8.9 | 59.0 ± 8.6 | 59.5 ± 8.4 | 59.6 ± 8.4 | 0.047 |
| LVESD, mm | 5231 | 49.8 ± 9.4 | 49.8 ± 8.8 | 49.9 ± 9.8 | 49.6 ± 8.7 | 49.9 ± 8.8 | 50.2 ± 8.6 | 0.530 |
| LA diameter, mm | 5194 | 42.5 ± 8.2 | 42.5 ± 7.4 | 42.8 ± 7.7 | 42.4 ± 7.7 | 42.3 ± 7.6 | 42.5 ± 7.9 | 0.719 |
| MR severity | 5236 | | | | | | | 0.133 |
| Severe | | 47 (5.9) | 44 (5.9) | 92 (8.7) | 92 (7.7) | 81 (9.5) | 49 (8.2) | |
| Moderate | | 214 (26.8) | 201 (27.0) | 266 (25.1) | 307 (25.8) | 221 (26.0) | 146 (24.4) | |
| Mild | | 440 (55.1) | 411 (55.2) | 590 (55.8) | 660 (55.6) | 457 (53.8) | 348 (58.1) | |
| Trivial/None | | 89 (11.2) | 79 (10.6) | 96 (9.1) | 122 (10.3) | 82 (9.7) | 51 (8.5) | |
| Follow up duration, month | 5236 | 46.4 ± 36.4 | 47.4 ± 38.4 | 48.4 ± 36.8 | 49.8 ± 38.2 | 49.7 ± 39.1 | 52.6 ± 40.3 | 0.001 |

Data are presented as frequencies (percentages) or means ± standard deviations. Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HF, heart failure; BNP, B-type natriuretic peptide; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; AST, aspartate aminotransferase; ALT, alanine amino transferase; LDL-C, low-density lipoprotein cholesterol; WBC, white blood cell; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic diameter; LA, left atrium; MR, mitral regurgitation; ICU, intensive care unit; PCI, percutaneous coronary intervention.

**Table 2.** Medications and in-hospital events of patients grouped by changes in heart rate from the admission day to the 90th day after discharge.

| Variable | n | Decrease ≥30 (n = 798) | Decrease 20–29 (n = 744) | Decrease 10–19 (n = 1058) | Decrease <10 (n = 1188) | Increase 1–10 (n = 849) | Increase >10 (n = 599) | p Trend |
|---|---|---|---|---|---|---|---|---|
| Medication for heart failure during the index admission | | | | | | | | |
| ARNI | 5236 | 18 (2.3) | 19 (2.6) | 27 (2.6) | 23 (1.9) | 27 (3.2) | 13 (2.2) | 0.811 |
| ACEI/ARB | 5236 | 719 (90.1) | 651 (87.5) | 916 (86.6) | 995 (83.8) | 714 (84.1) | 525 (87.6) | 0.004 |
| Beta-blocker | 5236 | 693 (86.8) | 648 (87.1) | 879 (83.1) | 953 (80.2) | 637 (75.0) | 468 (78.1) | <0.001 |
| Ivabradine | 5236 | 146 (18.3) | 71 (9.5) | 86 (8.1) | 59 (5.0) | 36 (4.2) | 25 (4.2) | <0.001 |
| MRAs | 5236 | 386 (48.4) | 317 (42.6) | 462 (43.7) | 459 (38.6) | 308 (36.3) | 207 (34.6) | <0.001 |
| Loop diuretics | 5236 | 725 (90.9) | 648 (87.1) | 895 (84.6) | 965 (81.2) | 685 (80.7) | 488 (81.5) | <0.001 |
| Digoxin | 5236 | 145 (18.2) | 100 (13.4) | 147 (13.9) | 177 (14.9) | 121 (14.3) | 94 (15.7) | 0.325 |
| Other medication during the index admission | | | | | | | | |
| DHP-CCB | 5236 | 302 (37.8) | 293 (39.4) | 437 (41.3) | 433 (36.4) | 283 (33.3) | 211 (35.2) | 0.010 |
| Amiodarone | 5236 | 70 (8.8) | 42 (5.6) | 64 (6.0) | 66 (5.6) | 38 (4.5) | 26 (4.3) | <0.001 |
| Oral hypoglycemic agents | 5236 | 266 (33.3) | 307 (41.3) | 432 (40.8) | 489 (41.2) | 343 (40.4) | 237 (39.6) | 0.032 |
| Insulin | 5236 | 266 (33.3) | 238 (32.0) | 352 (33.3) | 346 (29.1) | 240 (28.3) | 186 (31.1) | 0.029 |
| Statin | 5236 | 363 (45.5) | 356 (47.8) | 518 (49.0) | 595 (50.1) | 371 (43.7) | 274 (45.7) | 0.579 |
| Aspirin | 5236 | 538 (67.4) | 523 (70.3) | 760 (71.8) | 873 (73.5) | 596 (70.2) | 418 (69.8) | 0.239 |
| P2Y12 | 5236 | 423 (53.0) | 400 (53.8) | 586 (55.4) | 623 (52.4) | 434 (51.1) | 303 (50.6) | 0.133 |
| In-hospital event | | | | | | | | |
| Hospital days | 5236 | 15.1 ± 11.6 | 14.2 ± 12.2 | 13.8 ± 14.5 | 11.8 ± 10.4 | 12.1 ± 15.4 | 11.9 ± 10.9 | <0.001 |
| ICU days | 5236 | 3.0 ± 4.6 | 2.0 ± 3.8 | 2.0 ± 4.1 | 1.4 ± 3.4 | 1.1 ± 2.9 | 1.2 ± 3.0 | <0.001 |
| Shock | 5236 | 185 (23.2) | 111 (14.9) | 169 (16.0) | 153 (12.9) | 102 (12.0) | 91 (15.2) | <0.001 |
| Intubation | 5236 | 34 (4.3) | 22 (3.0) | 38 (3.6) | 24 (2.0) | 10 (1.2) | 10 (1.7) | <0.001 |
| Acute coronary syndrome | 5236 | 217 (27.2) | 166 (22.3) | 232 (21.9) | 229 (19.3) | 140 (16.5) | 102 (17.0) | <0.001 |
| PCI | 5236 | 133 (16.7) | 142 (19.1) | 184 (17.4) | 196 (16.5) | 148 (17.4) | 92 (15.4) | 0.343 |
| Medication for heart failure within 3 months after discharge | | | | | | | | |
| Beta-blocker | 5236 | 609 (76.3) | 559 (75.1) | 706 (66.7) | 748 (63.0) | 515 (60.7) | 340 (56.8) | <0.001 |
| Ivabradine | 5236 | 113 (14.2) | 58 (7.8) | 61 (5.8) | 51 (4.3) | 33 (3.9) | 23 (3.8) | <0.001 |
| Digoxin | 5236 | 109 (13.7) | 70 (9.4) | 105 (9.9) | 121 (10.2) | 82 (9.7) | 78 (13.0) | 0.535 |
| ACEi/ARB | 5236 | 604 (75.7) | 539 (72.4) | 715 (67.6) | 790 (66.5) | 564 (66.4) | 411 (68.6) | <0.001 |
| ARNI | 5236 | 16 (2.0) | 14 (1.9) | 24 (2.3) | 16 (1.3) | 23 (2.7) | 11 (1.8) | 0.876 |
| MRAs | 5236 | 321 (40.2) | 260 (34.9) | 366 (34.6) | 363 (30.6) | 234 (27.6) | 163 (27.2) | <0.001 |
| Loop diuretics | 5236 | 554 (69.4) | 520 (69.9) | 687 (64.9) | 756 (63.6) | 523 (61.6) | 366 (61.1) | <0.001 |

Data are presented as frequencies (percentages) or means ± standard deviations. Abbreviations: ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor blockers; MRAs, mineralocorticoid receptor antagonists; P2Y$_{12}$, purinergic receptor P2Y, G-protein coupled, 12.

## 2.5. Statistical Analysis

We categorized each patient into one of six ordinal groups on the basis of change in their heart rate from discharge to the 3-month screening period (decrease of ≥30 bpm, decrease of 20–29 bpm, decrease of 10–19 bpm, decrease of 0–9 bpm, increase of 1–10 bpm, and increase of >10 bpm). The associations among the baseline characteristics of the patients in the groups were tested using the Cochran–Armitage test for categorical variables, the general linear model for continuous variables, and the Jonckheere–Terpstra test for obviously skewed data (e.g., B-type natriuretic peptide levels). The association between the changes in the patients' heart rates and their risk of outcomes was assessed using a Cox proportional-hazards model. The linear trend across the ordinal groups on the risk of outcomes was tested. In addition, we obtained the hazard ratios and corresponding confidence intervals using the ≥30-beats-per-minute decrease group as the reference group. We adjusted for all the covariates listed in Tables 1 and 2 except the follow-up duration, including baseline heart rate and heart failure medications taken within 3 months of discharge, in the multivariable model.

Since the cut-off values used to group the patients were subjective and arbitrary, we explored the possibility of a nonlinear relationship between changes in heart rate and risk of outcomes by treating heart rate reduction as a flexible restricted cubic spline. The locations of knots were set to the 5th, 35th, 65th, and 95th percentiles. We adjusted for

the covariates in the restricted cubic spline model. Since our data set had some missing values, the Cox models (including the restricted cubic spline model) were calculated using the complete data after single expectation–maximization imputation. R (version 4.0.4, R Project for Statistical Computing) and the "rms" package (version 5.1 to 3.1) were used to generate the restricted cubic spline model. SAS (version 9.4, SAS Institute) was used for other statistical analyses. A two-sided *p*-value <0.05 was considered statistically significant.

## 3. Results

### 3.1. Patient Characteristics and Baseline Demographics

A total of 5236 patients were eligible in our analysis. The mean ($\pm$ standard deviation) age was 63.0 $\pm$ 15.5 years, and nearly 70% of the patients were male. Of note, 15.9% of the subjects had been admitted for heart failure in the previous year. The most prevalent comorbidity was hypertension (67.4%), followed by coronary artery disease (57.3%), diabetes (48.2%), dyslipidemia (42.3%), and chronic kidney disease (39.7%). The most common medications prescribed for heart failure during the index admission were ACEIs/ARBs (86.3%), loop diuretics (84.1%), and beta-blockers (81.7%). The mean baseline LVEF was 30.2 $\pm$ 7.2%, and about one-thirds (33.6%) of the patients had moderate or severe mitral regurgitation. The mean hospital days was 13.1 $\pm$ 12.7 days, and the mean ICU duration was 1.8 $\pm$ 3.7 days. During the 3-month screening period after discharge, the most common medications prescribed for heart failure were ACEIs/ARBs (69.2%), beta-blockers (66.4%), and loop diuretics (65%). Of note, the average follow-up duration was 49.0 $\pm$ 38.1 months.

The results of patients' characteristics and baseline demographics are listed in Tables 1 and 2. More than half of the patients had diagnosed coronary artery disease (51.9% to 61.2%) and hypertension (63.4% to 69.2%), and 39.8% to 50.6% of the patients had diabetes mellitus. Most (74.1% to 86.1%) had no records of previous HHFs in the 3 years preceding the index admission. Most of the patients received standard treatments, including ACEIs/ARBs (83.8% to 90.1%) and beta blockers (75.0% to 86.8%), during the index hospitalization. Most (80.7% to 90.9%) of the patients were prescribed loop diuretics. The mean LVEFs of the different groups ranged from 29.1 $\pm$ 7.7% to 30.6 $\pm$ 7.0%. The patients' hospital stay ranged from 11.9 $\pm$ 10.9 to 15.1 $\pm$ 11.6 days, of which the ICU constituted 1.2 $\pm$ 3.0 to 3.0 $\pm$ 4.6 days. In both cases, the most days were spent in the $\geq$30-bpm decrease group. Some of the patients experienced episodes of shock (12.0% to 23.2%) or respiratory failure (1.2% to 4.3%) during the index hospitalization. Heart failure medication coverage at the 3-month screening period was lower than that at admission (56.8–76.3% vs. 78.1–86.8% for beta blockers, and 66.4–75.7% vs. 87.6–90.1% for ACEIs/ARBs).

### 3.2. Changes in Heart Rate by the 3-Month Screening Period and Long-Term Outcomes

The primary outcome was the composite of HHF and cardiovascular death. The secondary outcomes were all-cause death, cardiovascular death, and HHF. The occurrences of the outcomes in each of the heart rate reduction groups are illustrated in Supplemental Table S1. According to the unadjusted Model 1, the occurrences of composite events increased significantly from the 10- to 19-bpm decrease group to the >10-bpm increase group (*p* for linear trend < 0.001, Model 1 in Table 3). The HHF also exhibited benefits among the patients' whose heart rates decreased by $\geq$20 bpm (*p* for linear trend < 0.001). With adjustment for all the covariates except heart failure medications taken within 3 months of discharge, the model revealed significant dose–response relationships between heart rate reduction and the four outcomes of interest. The results indicate that smaller decreases in heart rates from discharge to 3-month screening period were associated with less favorable prognoses (a higher risk of all outcomes; *p* for linear trend < 0.05, Model 2 in Table 3). The results remained unchanged when we adjusted for heart failure medications taken within 3 months of discharge (*p* for linear trend < 0.05, Model 3 in Table 3).

**Table 3.** Outcomes of patients grouped by changes in heart rate.

| Outcome | Decrease ≥30 (n = 798) | Decrease 20–29 (n = 744) | Decrease 10–19 (n = 1058) | Decrease <10 (n = 1188) | Increase 1–10 (n = 849) | Increase >10 (n = 599) | p Trend |
|---|---|---|---|---|---|---|---|
| Composite of heart failure hospitalization and cardiovascular death | | | | | | | |
| Model 1 | Ref | 1.08 (0.94–1.24) | 1.15 (1.01–1.30) * | 1.15 (1.02–1.30) * | 1.25 (1.10–1.42)* | 1.24 (1.07–1.42) * | <0.001 |
| Model 2 | Ref | 1.05 (0.91–1.22) | 1.12 (0.97–1.30) | 1.14 (0.97–1.32) | 1.23 (1.04–1.45)* | 1.25 (1.05–1.50) * | 0.003 |
| Model 3 | Ref | 1.05 (0.91–1.22) | 1.12 (0.96–1.29) | 1.13 (0.97–1.31) | 1.22 (1.03–1.44)* | 1.24 (1.03–1.48) * | 0.006 |
| Cardiovascular death | | | | | | | |
| Model 1 | Ref | 1.03 (0.84–1.26) | 1.19 (0.99–1.42) | 1.09 (0.92–1.31) | 1.12 (0.93–1.36) | 1.07 (0.87–1.32) | 0.404 |
| Model 2 | Ref | 1.01 (0.81–1.26) | 1.30 (1.05–1.61) * | 1.29 (1.03–1.62) * | 1.31 (1.03–1.67) * | 1.33 (1.02–1.73) * | 0.012 |
| Model 3 | Ref | 1.01 (0.81–1.25) | 1.27 (1.02–1.57) * | 1.25 (0.998–1.57) | 1.27 (0.998–1.63) | 1.27 (0.97–1.66) | 0.033 |
| Heart failure hospitalization | | | | | | | |
| Model 1 | Ref | 1.08 (0.93–1.26) | 1.16 (1.01–1.33) * | 1.19 (1.04–1.36) * | 1.27 (1.10–1.47) * | 1.26 (1.08–1.47) * | <0.001 |
| Model 2 | Ref | 1.07 (0.90–1.26) | 1.12 (0.95–1.32) | 1.13 (0.95–1.34) | 1.20 (1.002–1.45) * | 1.23 (1.003–1.50) * | 0.027 |
| Model 3 | Ref | 1.07 (0.91–1.27) | 1.12 (0.95–1.32) | 1.12 (0.94–1.33) | 1.20 (0.99–1.44) | 1.22 (0.99–1.49) | 0.042 |
| All-cause mortality | | | | | | | |
| Model 1 | Ref | 1.15 (0.98–1.33) | 1.11 (0.96–1.27) | 1.13 (0.98–1.30) | 1.12 (0.97–1.30) | 1.14 (0.97–1.33) | 0.197 |
| Model 2 | Ref | 1.12 (0.95–1.32) | 1.19 (1.01–1.41)* | 1.33 (1.11–1.58) * | 1.29 (1.07–1.56) * | 1.38 (1.12–1.69) * | 0.001 |
| Model 3 | Ref | 1.11 (0.94–1.31) | 1.17 (0.99–1.39) | 1.30 (1.09–1.55) * | 1.26 (1.05–1.53) * | 1.34 (1.09–1.64) * | 0.003 |

* $p$ <0.05; Model 1: unadjusted; Model 2: adjusted for all covariates (number of covariates = 54) listed in Tables 1 and 2, except follow-up duration, heart failure medications during the admission, and heart failure medications within 3 months after discharge; Model 3: adjusted for all the covariates listed in Tables 1 and 2 (number of covariates = 68), except follow-up duration.

The adjusted (fitted) survival rates of the patients are illustrated in Figure 2A–D. The possibility of a nonlinear relationship between heart rate reduction and risk of outcomes was further explored using the restricted cubic spline model. The results indicate that the relationship between heart rate reduction and risk of outcomes was linear $p$ for nonlinearity > 0.2, Figure 3A–D). We also evaluated the association between heart rate at the 3-month screening period and risk of outcomes (Supplemental Tables S2–S4). Unsurprisingly, a higher heart rate was significantly associated with less favorable outcomes.
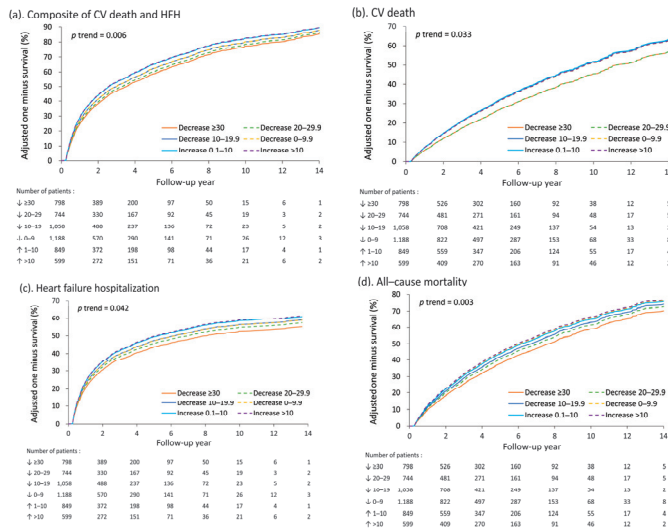


**Figure 2.** Adjusted (fitted) one minus survival for the composite outcome of cardiovascular death and HHF (**a**), cardiovascular death (**b**), HHF (**c**), and all-cause mortality (**d**) among patients with different changes in heart rate from discharge to 3-month screening period. HHF, hospitalization for heart failure; CV, cardiovascular.
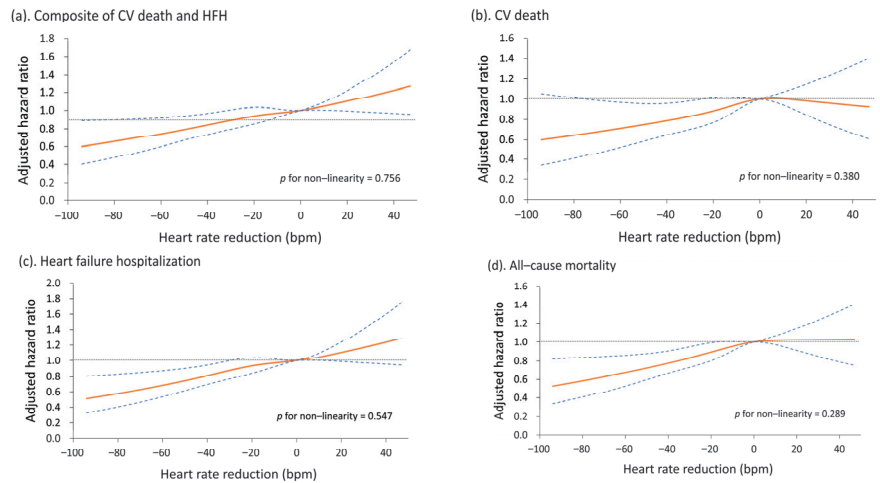
**Figure 3.** Relationship between changes in heart rate from discharge to 3-month screening period and risk of the composite outcome of cardiovascular death and HHF (**a**), cardiovascular death (**b**), HHF (**c**), and all-cause mortality (**d**) in patients with heart failure with reduced ejection fraction. HHF, hospitalization for heart failure; CV, cardiovascular.

## 4. Discussion

We analyzed the long-term outcomes of patients with heart failure requiring hospitalization whose heart rates changed by various degrees during the study period. The results of this study indicate that optimal heart rate control can help patients with HFrEF avoid cardiovascular death, HHF, and all-cause mortality in the long term.

Our study demonstrated that heart rate reduction strategies may influence the long-term outcomes of patients with HFrEF. The patients whose heart rates decreased by ≥20 bpm after discharge (relative to their baseline heart rate at admission) had significantly more favorable prognoses. The results were consistent after we adjusted for the patients' baseline characteristics and heart failure medications. Heart rate has often served as a monitoring target or predictive factor in studies on heart failure treatment [12]. A higher heart rate may indicate a more unstable condition. Analyses of data from European registries have revealed that patients have elevated heart rates when experiencing acute heart failure requiring admission [19,20]. However, the prognostic value of heart rate in acute heart failure remains controversial. One trial that enrolled patients hospitalized for acute heart failure identified baseline heart rate as a predictive factor for short-term adverse events [21]; however, Bertomeu-Gonzalez et al. observed that higher sinus rhythm heart rates at admission were not significantly associated with mortality [22]. Studies on the results of the Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study with Tolvaptan (EVEREST) trial revealed that heart rate at admission is not correlated with long-term all-cause mortality in patients with HFrEF in sinus rhythm [20,23]. A higher baseline heart rate may be an indicator of sympathetic overactivity, greater oxygen consumption, a lower myocardial coronary perfusion time, and endothelial inflammation [7,24]. Nevertheless, previous literature has suggested that baseline heart rate alone is an insufficient prognostic indicator for patients with heart failure.

Kurgansky et al. enrolled 51,194 patients with HFrEF with an LVEF of ≤35% in sinus rhythm from the US Veterans Affairs healthcare system. They discovered that a higher heart rate, both at the time of diagnosis and during follow-up, was strongly associated with an increased risk of adverse outcomes, [25] independent of the use of beta blockers. However, the results of our study indicate that the change between follow-up and baseline heart rate is more important. Our study differed in some respects from the large cohort study of Kurgansky et al. First, we enrolled patients hospitalized for HFrEF; therefore,

the cardiovascular symptoms experienced by the patients may have been more severe. The higher MRA and loop diuretic use rates in our study also indicated the severity of patients with decompensated HFrEF. In addition, we enrolled patients with basal heart rates of $\geq$70 bpm, and excluded those with atrial fibrillation or atrial flutter. Another study conducted by Kotecha et al. revealed that using ß-blockers reduces mortality in patients of sinus rhythm with heart failure, irrespective of resting heart rate. Patients with heart rate <70 bpm were also enrolled in this meta-analysis [26]. Similarly to Kurgansky's research, a higher resting heart rate at baseline and during follow-up increases mortality; however, patients without beta-blocker treatment experienced higher cardiac events. Proper treatment of heart rate for patients with heart failure of sinus rhythm could be beneficial. Our study also directed to the necessity of heart rate control. The beta-blocker usage rate at 3 months was highest in the $\geq$30-beats-per-minute decrease group, revealing a better long-term prognosis. However, the mean baseline heart rate of the $\geq$30-beats-per-minute decrease group was significantly higher than the mean across all the groups (113.9 bpm, $p < 0.001$). This group had more favorable long-term prognoses, including lower rates of mortality and HHF. The results suggest that the heart rate decreases in the 3 months after they began treatment was more important than the baseline heart rate of a patient with HFrEF. The benefits of heart rate reduction remained significant, even after adjustment for baseline heart rate, age, ejection fraction, heart failure medications, and other covariates.

Heart rate control has been used as a treatment modality for heart failure for decades. As the standard treatment, beta blockers lower a patient's heart rate, improve their sympathetic tone, reduce myocardial oxygen consumption, and control arrhythmia, resulting in more favorable clinical prognoses [13]. The beneficial effects of beta blockers strongly depend on their heart rate-reducing properties [27,28]. Ivabradine can be used to further reduce the heart rates of patients with HFrEF with sinus rhythm heart rates over 70 bpm, and help such patients achieve more favorable clinical outcomes, including a lower risk of mortality, especially when a patient's heart rate can be reduced by $\geq$15 bpm [12,29]. One post hoc analysis of the EVEREST trial revealed that heart rates of $\geq$70 bpm after discharge are associated with an increased risk of mortality [23]. Nevertheless, heart rate reduction targets have rarely been discussed in the literature. Using our unadjusted models, we determined that a heart rate reduction of $\geq$20 bpm has significant benefits in terms of preventing the composite outcome of HHF and cardiovascular death. After adjustment for all covariates, the benefits remained significant. The overall mortality rate of the patients whose heart rates decreased by $\geq$30 bpm was significantly lower than that of the patients whose heart rates decreased by <10 bpm.

Compared with the patients enrolled in a previous study of registry data, [30] the patients in our study received more guideline-directed treatments during the index admission period, including beta blockers (78.1–86.8%), ACEIs/ARBs (83.8–90.1%), and MRAs (34.6–48.4%). During the follow-up period, the medication coverage rates (for beta blockers, ACEIs/ARBs, and MRAs) decreased. ACEIs/ARBs or MRAs may have been discontinued in our study because of hypotension or impaired renal function, since patients were with poor LV systolic function (EF 29.1–30.6%) or impaired renal function (Cr 2.0–2.2 mg/dL, eGFR 58–63). Beta blockers can have negative inotropic effects on cardiovascular hemodynamics, which causes many physicians to hesitate to prescribe or increase the dosage of such medications. Some physicians may change their patients' prescriptions from beta blockers to other agents or discontinue beta blockers because their patients are intolerant to such medications. Finally, the $\geq$30-bpm decrease group had the fewest long-term cardiac events, but had the lowest mean LVEF (29.1 $\pm$ 7.7%, $p < 0.001$), highest mean initial heart rate (113.9 $\pm$ 18.0 bpm, $p < 0.001$), longest mean hospital and ICU stays, and highest incidence of shock events during the index admission period. These patients also had higher coverage rates of guideline-directed medications, including ACEIs/ARBs, beta blockers, ivabradine, and MRAs, during the follow-up period. The better guideline-directed medications coverage rate may be another reason for why this group achieved more favorable outcomes than did the other groups. However, after adjustment for all the covariates,

including heart failure medications, greater heart rate reductions were still significantly associated with more favorable outcomes, including lower rates of overall mortality, and a lower incidence in the composite outcome of HHF and cardiovascular death. Our results are similar to those of a study by Hamill et al. that indicated that time-updated heart rates are more strongly related with cardiovascular outcomes than are baseline heart rates [31]. In the present study, only 20% of the patients (1062 of the 5236) had heart rates of <70 bpm at the 3-month screening period, indicating that heart rate management is often overlooked in the treatment of patients with chronic stable heart failure. Our study highlights the need to draw attention to this problem in the medical community, and to encourage early adoption of heart rate-lowering treatment strategies.

## 5. Limitations

Although this study provides key insights into the long-term clinical outcomes of heart rate control in patients with heart failure after hospitalization, it has some limitations. Firstly, because of the retrospective nature of this study, the different heart rate groups may have had inherent differences. The retrospective design also limited our ability to enroll patients randomly, and may have caused selection bias. Patients' underlying conditions could have also altered the heart rate, including infection, inflammation, bleeding, or sepsis. Therefore, in our analysis, we adjusted for all the available covariates that may have been related to the outcomes. Secondly, heart rate was a key parameter in this study; however, data related to daily variations in the patients' heart rates during follow-up were not collected. Heart rate from the in-hospital Holter devices would perhaps have been more reflective of the actual state. However, patients admitted with decompensated HFrEF seldom received Holter for heart rate recording in daily practice. This is also the limitation of the real-world retrospective analysis. Furthermore, the heart rate at admission was the condition before adequate and proper treatment. We recorded the heart rate upon admission as the baseline to compare; however, it could have been overestimated. Thirdly, undertaking physical activity and rehabilitation programs after acute exacerbations of heart failure may strongly affect a patient's prognosis; however, information on the patients' daily physical activity habits or rehabilitation statuses were unavailable in our database. Furthermore, this study only included patients in sinus rhythm; therefore, the effect of heart rate control on patients with atrial fibrillation still warrants further investigation. Finally, medication noncompliance may have occurred, and the information we obtained on the drugs prescribed to the patients may not have reflected the patients' actual use of the drugs.

## 6. Conclusions

In this study, greater reductions in heart rate from discharge until the 3-month screening period were associated with a lower incidence of cardiovascular death, HHF, and all-cause mortality among patients discharged after hospitalization for decompensated HFrEF. Researchers should comprehensively evaluate guideline-directed therapies to determine which is most effective in helping patients achieve a target heart rate reduction and, in turn, more favorable long-term prognoses.

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MDPI

*Review*

# Urinary microRNA in Diabetic Kidney Disease: A Literature Review

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**Abstract:** Diabetic kidney disease is the most common primary disease of end-stage kidney disease globally; however, a sensitive and accurate biomarker to predict this disease remains awaited. microRNAs are endogenous single-stranded noncoding RNAs that have intervened in different post-transcriptional regulations of various cellular biological functions. Previous literatures have reported its potential role in the pathophysiology of diabetic kidney disease, including regulation of Transforming Growth Factor-β1-mediated fibrosis, extracellular matrix and cell adhesion proteins, cellular hypertrophy, growth factor, cytokine production, and redox system activation. Urinary microRNAs have emerged as a novel, non-invasive liquid biopsy for disease diagnosis. In this review, we describe the available experimental and clinical evidence of urinary microRNA in the context of diabetic kidney disease and discuss the future application of microRNA in routine practice.

**Keywords:** diabetes mellitus; diabetic kidney disease; exosomes; microRNA; urinary

Diabetes mellitus (DM) is a major health threat involving 463 million persons globally [1,2]. Diabetic kidney disease (DKD) constitutes the top cause of end-stage kidney disease worldwide [3,4]. The medical burden is complicated by its strong association with cardiovascular diseases, death [5,6], as well as elevated medical financial demand [7]. Improvements in the understanding of pathophysiology and early prediction of DKD remain an unmet clinical need.

Knowledge of systems biology and advances in high-throughput sequencing technology have revolutionized the understanding of the pathophysiology of DKD and biomarker development [8]. Clinical translation of different omic biomarkers for the prediction of DKD remains limited in terms of sample size and validation cohort. An approach that precisely identifies predisposed high-risk DM patients to renal progression is urgently awaited.

Optimal biomarkers have to provide adequate sensitivity and specificity, be obtainable in a non-invasive nature and assay with friendly laboratory skills using minimal time and economical consumption. Urine represents an ideal non-invasive biomarker, mainly for genitourinary diseases, because it is easily collected in large quantities without injury to the patient. In addition to varied types of proteins, the exosomes, which can be secreted by cells of different nephron segments, may also transport protein, mRNA, and microRNA (miRNA) markers produced in conditions of kidney malfunction or structural damage [9]. From them, the urinary exosomes can provide a panoramic view of the whole urinary system.

In this narrative literature review, we examine emerging evidence from experimental and human research that suggest the potential role of urinary miRNA for clinical application in the context of DKD.

### 1. Introduction of microRNA

The miRNAs are important epigenetic regulators of gene expression that intervene in various cellular processes of health and disease status. Genes encoding miRNAs are situated in the noncoding region or in introns of either protein-coding genes (miR-trons) or noncoding RNA [10]. These are small, less than 70 nucleotide stem–looped structures transcribed by RNA polymerase II located in the nucleus. miRNAs rarely bind to the coding regions of mRNA or genomic DNA, including promoter regions. However, they can actively play a role as regulators of cellular crosstalk by modifying their transcriptional program [11]. Currently, there are more than 38,000 mature sequences of miRNA included in miRbase, and the entry list is still growing [12].

Most miRNAs remain stable and have a prolonged half-life, but other individual miRNAs can experience a rapid decline in certain cellular circumstances because of the existence of specific environmental stimuli or cellular factors [13]. miRNAs are ubiquitous and can be present in different compartments of the body, including blood, urine and other body fluids. To avoid the degradation of miRNA by ribonucleases, they are often packaged in the form of micro-vesicles or exosomes or carried by RNA-binding proteins [14]. The miRNAs are potentially superior biomarkers than proteins and mRNAs because of their stability in body fluids and the high reproducibility by using accurate and sensitive amplification methods. However, the isolation and quantification of miRNAs are technique and time laborious, which limits their applications in routine clinical practice.

### 2. miRNAs in Kidney Homeostasis

Several miRNAs are expressed primarily in the adult human kidney (such as miR-215, miR-146a and miR-886); other miRNAs (for example, miR-192, miR-194, miR-21, miR-200a, miR-204 and let-7a–g), are increased in the kidney as well as in other organs [15]. This expression is tissue-specific and can be dependent on the developmental stage. Deletion of the miR-17~92 cluster leads to defective embryogenesis, affecting progenitor cells and the development of nephrons. Mice deficient in miR-17~92 are categorized by renal hypodysplasia and cause glomerular damage and proteinuria [16].

miRNAs are involved in different renal cellular processes, glomerular haemodynamia, as well as the maintenance of fluid and electrolyte balance. Other miRNAs in the kidney can also affect renin-expressing juxtaglomerular cells, which leads to damage to these cells. Consequently, plasma renin level decreased, and it was associated with hypotension and renal fibrosis [17]. The miRNAs also can help in the osmolarity homeostasis and have effects on the process of $Na^+$, $K^+$ and $Ca^{2+}$ regulation in the condition of hypertonic environments [18].

### 3. Role miRNA in DKD: Murine Experiments

Hyperglycaemia can trigger a complex interplay between metabolic and hemodynamic factors leading to the genesis of various diabetic complications, including DKD. The presence of high glucose concentration has an adverse influence on all renal cell lineages (mesangial cells, tubular cells, podocytes and endothelial cells) and can modulate the expression of miRNAs affecting cellular intercommunication and kidney tissue homeostasis [19].

Numerous studies have demonstrated the difference in the expression of circulating miRNAs throughout the progression of DKD [15]. A high glucose condition affects the expression of miR-29a and miR-29c, leading to the apoptosis of podocytes and the promotion of pro-fibrotic substances [20–22]. The hyperglycemia also regulates the expression of miR-25, miR-93 and miR-192, which in turn affects the redox system, vascular endothelial growth factor and tubulointerstitial fibrosis [23–25]. A comprehensive description of changes in the expression of 41 miRNAs in different animal studies is summarized in Table 1.

**Table 1.** miRNA implicated in the diabetic animal model.

| Mature Sequence of miRNA | Species | Experimental Model | Expression | Number of Study | References |
|---|---|---|---|---|---|
| hsa-let-7i-5p | Mouse | Db mice | Up | 1 | [26] |
| hsa-miR-129-1-3p | Mouse | Db mice | Down | 1 | [26] |
| hsa-miR-130a-3p | Mouse, cell | Db mice, HepG2 cells | Up | 1 | [27] |
| hsa-miR-133b | Rat | High-Fat Diet/Streptozotocin-Induced Diabetic Rat | Up | 1 | [28] |
| hsa-miR-134-5p | Mouse, cell | C57BL/KsJ db mice, Db/Db mice, podocytes | Up | 1 | [29] |
| hsa-miR-146a-5p | Mouse, cell | C57BL/6J, miR-146a−/− mice, Podocytes, HK-2 human kidney cells, Db mice, peritoneal macrophages | Up, Down | 3 | [30–32] |
| hsa-miR-148b-3p | Rat, cell | Proximal tubular cells | Down | 1 | [33] |
| hsa-miR-16-5p | Rat | Male Wistar rats | Up | 1 | [34] |
| hsa-miR-181a-5p | Rat, cell | HK-2 cells, Otsuka-Long-Evans-Tokushima-Fatty rats | Down | 1 | [35] |
| hsa-miR-192-5p | Mouse | Db mice, Ets-1–deficient mice | Up | 1 | [36] |
| hsa-miR-200c-3p | Mouse, cell | Db mice, M4200 cells | Down | 3 | [26,37,38] |
| hsa-miR-21-5p | Mouse, rat, cell | C57BI/6J mice, miR-21-KO mice, DBA/2J mice, Db mice, kk-ay mice, epithelial-to-mesenchymal, podocytes | Up, Down | 7 | [39–45] |
| hsa-miR-217-5p | Cell | Glomerular mesangial cells | Up | 1 | [46] |
| hsa-miR-218-5p | Mouse, cell | Db mice, podocytes | Up, Down | 2 | [26,47] |
| hsa-miR-23c | Rat, cell | HK-2 cells | Down | 1 | [48] |
| hsa-miR-26a-5p | Rat, cell | OLETF rats, NRK-52E cells, glomerular, mesangial cells, podocytes, proximal tubular epithelial cells | Up, Down | 2 | [49,50] |
| hsa-miR-29a-3p | Mouse, cell | Db mice, Smad3-knockout (KO) db mice, mouse embryonic fibroblasts, NRK52E cells, mesangial cells | Down | 2 | [51,52] |
| hsa-miR-29a-5p | Mouse | Male FVB mice | Down | 1 | [53] |
| hsa-miR-29c-3p | Mouse | Db mice | Down | 1 | [26] |
| hsa-miR-30a-5p | Rat | High-Fat Diet/Streptozotocin-Induced Diabetic Rat | Up | 1 | [28] |
| hsa-miR-335-5p | Mouse | Male C57BL/6J mice | Up | 1 | [54] |
| hsa-miR-33a-5p | Mouse, cell | Db mice, MMCs (CRL1927) and HEK293 cells | Down | 1 | [55] |
| hsa-miR-342-5p | Rat | High-Fat Diet/Streptozotocin-Induced Diabetic Rat | Up | 1 | [28] |
| hsa-miR-34a-5p | Mouse, cell | Db mice, mesangial cells, HK-2cells, podocytes | Down | 3 | [56–58] |
| hsa-miR-375-5p | Mouse | Male C57BL/6J mice | Up | 1 | [54] |
| hsa-miR-378d | Mouse | Db mice | Up | 1 | [26] |
| hsa-miR-451a | Mouse, rat | Db mice, male Wistar rats | Down | 2 | [34,59] |
| hsa-miR-7977 | Mouse | Db mice | Down | 1 | [26] |
| hsa-miR-92b-5p | Rat | Lean Zucker, obese Zucker rats | Down | 1 | [60] |
| hsa-miR-93-5p | Mouse, cell | Pod-iCreERT2 mice, podocytes | Down | 1 | [61] |
| hsa-miR-99b-5p | Mouse, rat, cell | Db mice, mesangial cells, proximal tubular epithelial cells | Down | 4 | [62–65] |

miRNAs are annotated as a mature sequence of miRNA identified from the miRbase database.

#### 4. Urinary miRNA Research: Human Evidence

Research into miRNA has unveiled obscure puzzles of the pathophysiology of DKD. Furthermore, other investigators have interrogated the clinical utility of these miRNAs for routine practice. Emerging evidence indicates the probable roles of urinary miRNAs as predictors of DKD development or progression [66]. Urine miRNA levels provide a direct reflection of kidney tissue damage. These miRNAs originate from cells and are encapsulated into extracellular vesicles, named exosomes, and are secreted in various biological fluids, including urine. Exosomes can exert paracrine effects and serve as a mediator of intercellular communication. They are stable in biological fluid as well as in paraffin-embedded sections, rendering these exosomes suitable as "liquid biopsy" or biomarkers of specific disease conditions [67]. A number of studies have examined the relationship between urinary microRNA and blood sugar levels. They found that concentrations of various urinary extravesical miRNAs (such as miRNA-941-5p, miRNA 34c-5p and miRNA-208a-3p) were correlated with levels of glycosylated hemoglobin [68]. Table 2 lists changes in the expression of urinary miRNA associated with DKD in clinical research. We identified 141 unique urinary miRNAs associated with DKD. Peculiarly, the direction of expression of specific miRNA differs between types of DM (type 1 vs. 2) and also between distinct patient cohorts (as highlighted in bold). A possible explanation might reside in unclear mechanisms between the two types of DM. In addition, several technical concerns may affect the miRNA profiling, including methods of specimen collection (processing and storage), a fraction of extracted urine (urine, urinary extra-vesicle, extra-vesicle depleted urine fraction), analytic platform (qPCR, microarray, genome-wide profiling by small-RNA sequencing) and artifact contamination [68]. Finally, the cell source of urinary miRNA can arise from any genitourinary tract, and the function of urinary miRNA is not necessarily the same as circulating ones. Sophisticated bioinformatics analysis and network interaction maps are capable of identifying Gene Ontology processes, classification and relevant pathways of target genes in this modern era [66,69]. These approaches may help to decipher the biological functions of urinary miRNA in the pathophysiology of DKD.

**Table 2.** Urinary miRNA implicated in human studies related to diabetic kidney disease.

| Mature Sequence of miRNA | Sample | DM Type | Patient Number | Urinary Expression in DKD | References |
|---|---|---|---|---|---|
| has-let-7a-3p | Blood/Urine | 2 | 27 | Down | [66] |
| hsa-let-7c-5p | Urine | 2 | 63 | Up | [70] |
| hsa-let-7f-1-3p | Blood/Urine | 2 | 27 | Down | [66] |
| hsa-let-7i-5p | Urine | 2 | 160 | Up | [71] |
| hsa-miR-106b-3p | Blood/Urine | 2 | 27 | Down | [66] |
| hsa-miR-10a-5p | Urine | 1 | 48 | Up vs. PMA | [68] |
| hsa-miR-10b-5p | Urine | 1 | 48 | Up vs. PMA | [68] |
| hsa-miR-1224-3p | Urine | 1 | 40 | Up | [72] |
| hsa-miR-122-5p | Urine | 1 | 48 | Up | [68] |
| hsa-miR-126 | Urine | 2 | 92 | Up | [73] |
| hsa-miR-126-5p | Urine/blood | 1 | 147 | Down | [74] |
| hsa-miR-1275 | Urine | 2 | 6 | Up | [75] |
| hsa-miR-1307-3p | Urine | 1 | 48 | Down | [68] |
| hsa-miR-130a-3p | Urine | 1 | 48 | Up | [68] |
| hsa-miR-130a-5p | Tissue/Urine | 1 | 24 | Up | [76] |
| hsa-miR-133a-3p | Urine | 1 | 48 | Up | [68] |
| hsa-miR-133a-3p | Urine | 2 | 6 | Down | [77] |
| hsa-miR-133b | Urine | 2 | 220 | Up | [78] |
| hsa-miR-135b-5p | Urine | 2 | 160 | Up | [71] |
| hsa-miR-141-3p | Urine | 1 | 40 + 48 | Up | [68,72] |
| hsa-miR-142-3p | Urine | 1 | 48 | Up | [68] |
| hsa-miR-145-5p | Tissue/Urine | 1 | 24 | Up | [76] |
| hsa-miR-146 | Urine | 2 | 92 | Up | [73] |
| hsa-miR-148a-3p | Urine | 1 | 48 | Up | [68] |

**Table 2.** *Cont.*

| Mature Sequence of miRNA | Sample | DM Type | Patient Number | Urinary Expression in DKD | References |
|---|---|---|---|---|---|
| hsa-miR-148b-3p | Urine | 2 | 56 | Down | [79] |
| hsa-miR-150-3p | Urine | 2 | 6 | Up | [77] |
| hsa-miR-150-5p | Urine | 2 | 80 | Up | [80] |
| hsa-miR-152-3p | Urine | 1 | 48 | Up | [68] |
| hsa-miR-153-3 | Urine | 2 | 6 | Down | [77] |
| hsa-miR-155 | Urine | 2 | 92 | Up | [73] |
| hsa-miR-155-5p | Tissue/Urine | 1 | 24 | Down | [76] |
| hsa-miR-1587 | Urine | 2 | 6 | Up | [75] |
| hsa-miR-15a-5p | Urine | 2 | 80 | Down | [80] |
| hsa-miR-15b-5p | Urine | 2 | 232 + 63 + 160 | Up/Down/Down | [70,71,81] |
| **hsa-miR-17-5p** | **Urine** | **2** | **56** | **Down** | **[79]** |
| **hsa-miR-17-5p** | **Urine** | **1** | **40** | **Up** | **[72]** |
| hsa-miR-181a-5p | Urine | 1 | 48 | Up | [68] |
| hsa-miR-183-5p | Urine | 1 | 48 | Up | [68] |
| hsa-miR-188-3p | Urine | 1 | 40 | Down | [72] |
| **hsa-miR-188-5p** | **Urine** | **1** | **48** | **Down** | **[68]** |
| **hsa-miR-188-5p** | **Urine** | **2** | **6** | **Up** | **[77]** |
| hsa-miR-190a-5p | Blood/Urine | 2 | 27 | Down | [66] |
| hsa-miR-1912 | Urine | 1 | 40 | Up | [72] |
| hsa-miR-1913 | Urine | 1 | 40 | UP | [72] |
| **hsa-miR-192-5p** | **Urine** | **2** | **56** | **Down** | **[79]** |
| **hsa-miR-192-5p** | **Urine** | **1** | **48** | **Up** | **[68]** |
| hsa-miR-193b-5p | Urine | 2 | 6 | Down | [69] |
| hsa-miR-196a | Urine | 2 | 209 | UP | [82] |
| **hsa-miR-197-3p** | **Urine** | **2** | **160** | **Down** | **[71]** |
| **hsa-miR-197-3p** | **Urine** | **1** | **48** | **Down** | **[68]** |
| hsa-miR-200a-3p | Urine | 1 | 48 | Up | [68] |
| hsa-miR-200c-3p | Urine | 1 | 48 | Up | [68] |
| hsa-miR-2117 | Urine | 2 | 6 | Up | [75] |
| hsa-miR-214-3p | Urine | 1 | 40 | UP | [72] |
| **hsa-miR-21-5p** | **Urine** | **2** | **56** | **Down** | **[79]** |
| **hsa-miR-21-5p** | **Urine/blood** | **1** | **147** | **Up** | **[74]** |
| **hsa-miR-216a-5p** | **Urine** | **2** | **56** | **Down** | **[79]** |
| **hsa-miR-216a-5p** | **Urine** | **1** | **50** | **Down** | **[83]** |
| hsa-miR-217-5p | Urine | 2 | 56 | Down | [79] |
| hsa-miR-219a-3p | Urine | 2 | 6 | Up | [75] |
| hsa-miR-221-3p | Urine | 1 | 40 | Down | [72] |
| hsa-miR-222-3p | Urine | 1 | 48 + 40 | Up | [68,72] |
| hsa-miR-22-3p | Urine | 1 | 48 | Up | [68] |
| hsa-miR-23c | Urine | 2 | 6 | Up | [77] |
| hsa-miR-24-3p | Urine | 2 | 160 | Up | [71] |
| hsa-miR-27b-3p | Urine | 2 | 160 | Down | [71] |
| hsa-miR-29a-3p | Urine | 2 | 83 | NC | [84] |
| hsa-miR-29a-5p | Urine | 2 | 83 | Up | [84] |
| hsa-miR-29b-1-5p | Urine | 1 | 40 | Up | [72] |
| **hsa-miR-29c-3p** | **Urine** | **2** | **220 + 56 + 63** | **Up/Up/Down** | **[70,78,79]** |
| **hsa-miR-29c-3p** | **Urine** | **1** | **48** | **Up** | **[68]** |
| **hsa-miR-29c-5p** | **Urine** | **2** | **83** | **NC** | **[84]** |
| **hsa-miR-29c-5p** | **Tissue/Blood/Urine** | **2** | **27 + 16** | **Down** | **[66,85]** |
| hsa-miR-30a-3p | **Urine** | **2** | **160** | **Up** | **[71]** |
| **hsa-miR-30a-5p** | **Urine** | **1** | **48 + 27** | **Up/Down** | **[68,86]** |
| hsa-miR-30b-5p | Blood/Urine | 2 | 27 | Down | [66] |
| hsa-miR-30c-5p | Blood/Urine | **2** | **27** | **Down** | **[66]** |
| hsa-miR-30d-5p | Blood/Urine | 2 | 27 | Down | [66] |
| hsa-miR-30e-3p | Blood/Urine | 2 | 27 | Down | [66] |
| hsa-miR-3137 | Urine | 2 | 6 | Down | [69] |
| hsa-miR-31-5p | Urine | 1 | 48 | Up | [68] |
| hsa-miR-3168 | Urine | 1 | 48 | Down | [68] |

**Table 2.** *Cont.*

| Mature Sequence of miRNA | Sample | DM Type | Patient Number | Urinary Expression in DKD | References |
|---|---|---|---|---|---|
| hsa-miR-3184-3p | Urine | 1 | 48 | Up | [68] |
| hsa-miR-320b | Blood/Urine | 2 | 27 | Up | [66] |
| hsa-miR-320c | Urine | 2 | 41 | Up | [87] |
| hsa-miR-320e | Urine | 2 | 6 | Up | [77] |
| hsa-miR-323b-5p | Urine | 1 | 40 | Down | [72] |
| hsa-miR-331-3p | Blood/Urine | 2 | 27 | Down | [66] |
| hsa-miR-335-5p | Urine | 1 | 40 | Up | [72] |
| hsa-miR-339-3p | Urine | 1 | 48 | Up | [68] |
| hsa-miR-342-3p | Urine | 1 | 48 | Up | [68] |
| hsa-miR-342-5p | Urine | 2 | 220 | Up | [78] |
| hsa-miR-34a-5p | Urine | 2 | 232 | Up | [81] |
| hsa-miR-362-3p | Urine | 2 | 80 | Up | [80] |
| hsa-miR-362-5p | Urine | 1 | 48 | Down | [68] |
| hsa-miR-363-3p | Urine | 1 | 27 | Down | [86] |
| hsa-miR-3677-3p | Urine | 2 | 6 | Up | [77] |
| hsa-miR-373-5p | Urine | 1 | 40 | Down | [72] |
| hsa-miR-375-5p | Urine | 2 | 160 | Down | [71] |
| **hsa-miR-377-5p** | **Urine** | **1** | **50** | **Up** | **[83]** |
| **hsa-miR-377-5p** | **Urine** | **2** | **56** | **Up** | **[79]** |
| hsa-miR-424-3p | Urine | 1 | 48 | Down | [68] |
| **hsa-miR-424-5p** | **Tissue/Urine** | **1** | **40 + 24 + 27** | **Up/Down/Down** | **[72,76,86]** |
| hsa-miR-4270 | Urine | 2 | 6 | Up | [69] |
| hsa-miR-4286 | Urine | 1 | 48 | Down | [68] |
| hsa-miR-429 | Urine | 1 | 40 | UP | [72] |
| hsa-miR-433 | Urine | 1 | 40 | Up | [72] |
| hsa-miR-4491 | Urine | 2 | 6 | Up | [75] |
| hsa-miR-4507 | Urine | 2 | 6 | Up | [75] |
| hsa-miR-4516 | Urine | 2 | 6 | Up | [75] |
| hsa-mir-453 | Urine | 1 | 40 | Down (Up in persistent microalbuminuria) | [72] |
| hsa-miR-4534 | Urine | 2 | 6 | Up | [75] |
| hsa-miR-4687-3p | Urine | 2 | 6 | Up | [75] |
| hsa-miR-486-3p | Urine | 1 | 40 | Up | [72] |
| hsa-miR-486-5p | Urine | 1 | 27 | Up | [86] |
| hsa-miR-495-5p | Urine | 1 | 27 | Down | [86] |
| hsa-miR-498 | Urine | 2 | 6 | Up | [75] |
| hsa-miR-5007-3p | Urine | 2 | 6 | Up | [75] |
| hsa-miR-500a-5p | Urine | 2 | 160 | Down | [71] |
| hsa-miR-5088-5p | Urine | 2 | 6 | Up | [75] |
| hsa-miR-5091 | Urine | 2 | 6 | Up | [75] |
| hsa-miR-516b-5p, | Urine | 2 | 6 | Up | [75] |
| hsa-miR-520h | Urine | 1 | 40 | Down | [72] |
| hsa-miR-524-5p | Urine | 1 | 40 + 27 | Down/Down | [72,86] |
| hsa-miR-548ah-3p | Urine | 2 | 6 | Up | [77] |
| hsa-miR-548p | Urine | 2 | 6 | Up | [77] |
| hsa-miR-552-5p | Urine | 1 | 40 | Up | [72] |
| hsa-miR-589-5p | Urine | 1 | 40 | Down | [72] |
| hsa-miR-6068 | Urine | 2 | 41 | Up | [87] |
| hsa-miR-6076 | Urine | 2 | 41 | NC | [87] |
| hsa-miR-616-5p | Urine | 1 | 27 | Up | [86] |
| hsa-miR-619-5p | Urine | 1 | 40 | Up | [72] |
| hsa-miR-628-5p | Urine | 1 | 40 | Up | [72] |
| hsa-miR-636 | Urine | 2 | 232 | Up | [81] |
| hsa-miR-638 | Urine | 1 | 40 | UP | [72] |
| hsa-miR-640 | Urine | 1 | 27 | Up | [86] |
| hsa-miR-645 | Urine | 1 | 27 | Up | [86] |
| hsa-miR-665 | Urine | 1 | 27 | Down | [86] |
| hsa-miR-6809-5p | Urine | 2 | 6 | Down | [69] |

**Table 2.** *Cont.*

| Mature Sequence of miRNA | Sample | DM Type | Patient Number | Urinary Expression in DKD | References |
|---|---|---|---|---|---|
| hsa-miR-6831-5p | Urine | 2 | 6 | Up | [69] |
| hsa-miR-760 | Urine | 2 | 6 | Up | [77] |
| hsa-miR-765 | Urine | 1 | 40 | UP | [72] |
| hsa-miR-767-3p | Urine | 1 | 27 | Down | [86] |
| hsa-miR-770-5p | Urine | 1 | 27 | Up | [86] |
| hsa-miR-7846-3p | Urine | 2 | 6 | Up | [69] |
| hsa-miR-877-3p | Urine | 2 | 80 | Up | [80] |
| hsa-miR-92a-3p | Urine | 1 | 48 + 40 | Up/Down | [68,72] |
| hsa-miR-92b-5p | Urine | 1 | 40 | UP | [72] |
| hsa-miR-93-5p | Urine | 2 | 160 | Up | [71] |
| hsa-miR-98-3p | Blood/Urine | 2 | 27 | Down | [66] |
| **hsa-miR-99b-5p** | **Urine** | **1** | **48** | **Up** | **[68]** |
| **hsa-miR-99b-5p** | **Blood/Urine** | **2** | **27** | **Down** | **[66]** |

miRNAs are annotated as a mature sequence of miRNA identified from the miRbase database. NC: not changed. miRNA in bold indicates differentially expressed direction between publications.

## 5. Performances of Urinary miRNAs as Disease Biomarker

A body of literature attempted to identify potential biomarkers from urine specimens in predicting the degree of kidney damage. Individual miRNAs or a cluster of miRNAs were used as possible biomarkers of DKD with satisfactory prediction performance. Li et al. found that urinary expression of let-7c-5p, miR29c-5p and miR-15b-5p could predict DKD with the area under curves (AUC) of 0.818, 0.774, and 0.818, respectively [70]. Eissa S et al. observed that the AUC of miR-15b, miR-34a, and miR-636 were 0.883, 0.917 and 0.984, respectively, for distinguishing DKD from controls. The panel composed of three miRNAs yielded an AUC of 0.912 [78,81]. The expressed levels of miR-95-3p, miR-185-5p, miR-1246, and miR-631 in urinary sediments can also yield a good accuracy (AUC, 0.863) for differentiating DKD from non-DKD or other disease conditions [88]. Collectively, all this evidence suggests the potential use of urinary miRNAs as non-invasive biomarkers for predicting DKD.

## 6. Conclusions

Sensitive biomarkers to guide decision-making in the management of DKD remain urgently awaited. Urinary miRNA may represent promising non-invasive, and cheap means with diagnostic or predictive implications in DKD. However, the clinical application is unsatisfactory to date because of inconsistencies between reported data. Most of the research projects reported were discovery experiments with small samples and limitation validation. In addition, the discrepancies may be in part explained by differences in procedures of urine isolation, the proportion of urine fraction (fresh urine, concentrates of extra-vesicles or extra-vesicle depleted fraction) used, heterogeneity in reporting outcome and in the degree of kidney severity (micro vs. macroalbuminuria, intermittent vs. persistent proteinuria). Furthermore, the advances in analytic methodology enabled the discovery of new miRNAs. Recently, the introduction of the sensor-based methodology using labels on magnetic beads can magnify measurement power. The incorporation of the use of spectrophotometry or electrochemistry, rather than direct visualization, can further enhance the quantification accuracy [89] with small sample sizes and limited validation. Consensus and standardization of methodologies applied to retrieve, isolate, store and measure miRNAs may enhance the reproducibility of the study results. Further validation with an extended sample size is warranted to move the field forward clinical translation of urinary miRNA in DKD.

## 7. Review Criteria

We conducted a narrative review of animal and human studies in published literature from 1 October 2013 until 30 July 2022. The PubMed database was used for searching the scientific literature using the following search terms: "microRNA", "diabetic kidney disease", "diabetic nephropathy", and "urinary". This narrative literature review primarily focused on original articles written in the English language and published in peer-reviewed journals. The reference lists of included articles were also hand-searched. References were managed using EndNote 20.1.

Independent researchers (CKH and YTC) managed titles and abstracts to identify potentially eligible studies for full-text review. We only included original articles and case series with over two cases in the English language for further assessment. Data extraction was performed in duplicate by two independent reviewers (CCL, KJY). When multiple articles reporting data from the same study population were identified, the most comprehensive data were used. All reported miRNAs are indexed and annotated as a mature sequence of miRNA identified from the miRbase database. Studies were required to provide quantizable data on the expression levels of miRNA, such as fold changes (down-expression, up-expression, not change or absolute value) or area under curves. We contacted the study authors regarding possible incomplete data on miRNA quantities presented in selected publications. The methodological quality of included studies was not assessed in this narrative review because large heterogenicity presented in the testing techniques (ELISA, microarray, q-PCR, sequencing, etc.) and differences in reporting outcomes.

We identified 247 records from the PubMed database for the initial assessment, and only 63 articles were included for full-text review; 40 articles related to animal studies and 23 human research articles, respectively (Figure 1). This literature review included a thorough assessment of 41 miRNA from animal experiments and 141 miRNA from urine samples of DKD patients.
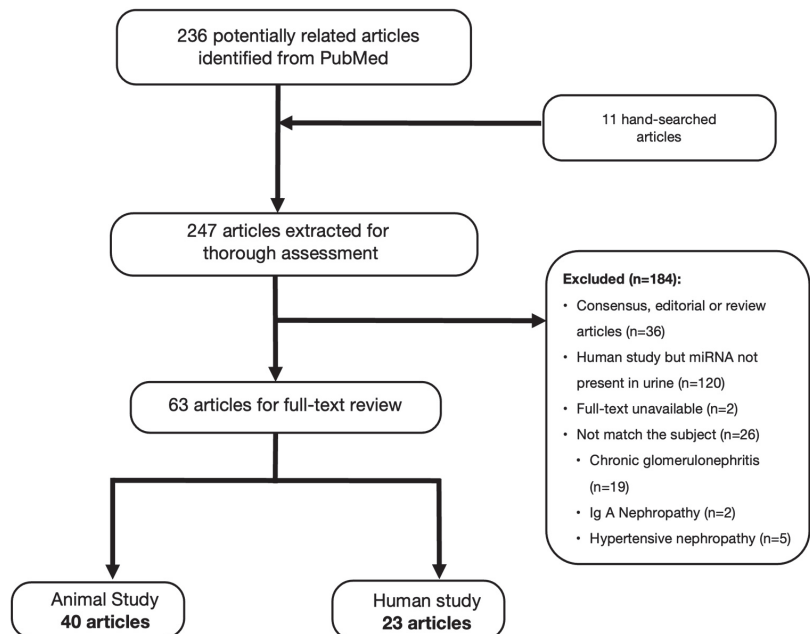
```
┌─────────────────────────────────┐
│ 236 potentially related articles│
│    identified from PubMed       │
└─────────────────────────────────┘
                 ↑      ┌──────────────────┐
                 ←──────│ 11 hand-searched │
                        │     articles     │
                        └──────────────────┘

┌─────────────────────────────────┐
│   247 articles extracted for    │
│      thorough assessment        │
└─────────────────────────────────┘
                 │         ┌─────────────────────────────────┐
                 ├────────→│ Excluded (n=184):               │
                 │         │ • Consensus, editorial or review│
                 │         │   articles (n=36)               │
                 │         │ • Human study but miRNA not     │
                 │         │   present in urine (n=120)      │
                 │         │ • Full-text unavailable (n=2)   │
┌─────────────────────────────┐   │ • Not match the subject (n=26) │
│ 63 articles for full-text   │   │   • Chronic glomerulonephritis │
│          review             │   │     (n=19)                      │
└─────────────────────────────┘   │   • Ig A Nephropathy (n=2)      │
                                  │   • Hypertensive nephropathy(n=5)│
                                  └─────────────────────────────────┘

┌──────────────────┐      ┌──────────────────┐
│  Animal Study    │      │   Human study    │
│   40 articles    │      │   23 articles    │
└──────────────────┘      └──────────────────┘
```

**Figure 1.** Flow chart of literature search and selection.

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# Associations of Three-Dimensional Anthropometric Body Surface Scanning Measurements and Coronary Artery Disease

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**Abstract:** *Background and Objectives*: The relationship between three-dimensional (3D) scanning-derived body surface measurements and biomarkers in patients with coronary artery disease (CAD) were assessed. *Methods and Methods*: The recruitment of 98 patients with CAD confirmed by cardiac catheterization and 98 non-CAD patients were performed between March 2016 and December 2017. A health questionnaire on basic information, life style variables, and past medical and family history was completed. 3D body surface measurements and biomarkers were obtained. Differences between the two groups were assessed and multivariable analysis performed. *Results*: It was found that chest width (odds ratio [OR] 0.761, 95% confidence interval [CI] = 0.586–0.987, $p = 0.0399$), right arm length (OR 0.743, 95% CI = 0.632–0.875, $p = 0.0004$), waist circumference (OR 1.119, 95% CI = 1.035–1.21, $p = 0.0048$), leptin (OR 1.443, 95% CI = 1.184–1.76, $p = 0.0003$), adiponectin (OR 0.978, 95% CI = 0.963–0.994, $p = 0.006$), and interleukin 6 (OR 1.181, 95% CI = 1.021–1.366, $p = 0.0254$) were significantly associated with CAD. The combination of biomarker scores and body measurement scores had the greatest area under the curve and best association with CAD (area under the curve of 0.8049 and 95% CI = 0.7440–0.8657). Conclusions: Our study suggests that 3D derived body surface measurements in combination with leptin, adiponectin, and interleukin 6 levels may direct us to those at risk of CAD, allowing a non-invasive approach to identifying high-risk patients.

**Keywords:** three-dimensional anthropometrics; waist circumference; chest width; adiponectin; leptin; coronary artery disease

## 1. Introduction

Cardiovascular disease, in particular coronary artery disease (CAD), is one of the main causes of morbidity and mortality in the world [1]. Obesity is described as an independent risk factor for CAD [2] and is generally defined by an excess of body fat with the most commonly used anthropometric index being the body mass index (BMI) [3]. Obese

individuals with the same amount of total body fat can have markedly distinct risk factor profiles [4], with abdominal fat having strong associations with CAD, mortality [5–8], and type 2 diabetes [9].

Visceral adipose tissue is an important endocrine organ, responsible for secreting hormones involved in a range of processes, e.g., control of sensitivity to insulin and inflammatory process mediators, and vascular hemostasis [10,11]. Biomarkers which play a role in insulin resistance and inflammation have been found to be associated with cardiovascular diseases. Leptin is an important link between obesity and the development of cardiovascular disease partially due to its effects on arterial pressure, formation of arterial thrombosis, aggregation of platelets, and on inflammatory vascular response [12]. Low adiponectin levels have also been found to be an independent risk factor for CAD [13]. The inflammatory biomarker C-reactive protein is positively correlated to the risk of cardiovascular events [14]. Interleukin 6 (IL6) and interleukin 8 have also been shown to play an important role in atherogenesis and atherosclerotic plaque destabilization [15,16]. It has also been demonstrated that the induction of the cytokine transforming growth factor beta-1 is associated with myocardial infarction [17].

A noninvasive three-dimensional (3D) scanning technology has been developed to obtain anthropometric measurements with many advantages over traditional methods, such as computed tomography scanners, X-rays, and bioelectrical impedance [18]. The aim of our study is to explore the association between CAD, biomarkers, and body measures with the use of 3D body scanning, providing more information to be used in clinical practice, epidemiological studies, and preventative medicine.

## 2. Materials and Methods

### 2.1. Study Subjects

From March 2016 to December 2017, a total of 98 patients found to have CAD as confirmed by cardiac catheterization exam at Chang Gung Memorial Hospital, Keelung, were recruited into our study CAD group. The same number of 98 sex- and age-matched patients presenting to our Department of Health Promotion and Examination were enrolled into the control group. Informed consent was obtained from all participants. This study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Review Board of Chang Gung Medical Foundation (201405148B0).

### 2.2. Anthropometrical Parameters

Three-dimensional body surface measurements were collected using a whole-body 3D laser scanner according to previously published methods. In addition to body weight, body height, and BMI, 35 measurements from four anatomical regions were made. The trunk region included the chest profile area, chest circumference, chest width (CW), waist profile area, waist circumference (WC), waist width, trunk volume, and trunk surface area. The head and neck region included the head surface area, head volume, head circumference, and neck circumference. The hip to the lower limb region included the hip profile area, hip circumference, hip width, left and right leg volume, left and right leg surface area, left and right calf circumference, left and right thigh circumference, and left and right leg length. The upper limb region included the left and right arm volume, left and right arm surface area, left and right arm length (RAL), left and right upper arm circumference, and left and right forearm circumference. The 3D laser scanning machine (LT3DCam) was built by Logistic Technology Company (LTC, Hsinchu, Taiwan), and was proven to have a high standard of accuracy due to the objective and comprehensive ways of measuring the human body surface. The standard procedure of measuring required the subject to remove all outer clothes except for underwear in preparation for scanning (women with bras in addition to pants) and to stand still on the stage for scanning (a total scanning time is about 10 s) [19]. The software system collected, realigned, constructed, and measured a subject's whole-body digital stature and selected information. The measurement error of the 3D

scanner in measuring the human body surface was checked; the error in the *x*- and *y*-axis was approximately 1 mm (1.2%), and in the *z*-axis it was less than 0.1 mm (0.2%) [20].

### 2.3. Data Collection

Upon recruitment, a questionnaire was given to acquire information on the following: date of birth; sex; occupation; education; marital status; history of cigarette smoking, alcohol drinking, and betel nut chewing; personal medical history (including hypertension, diabetes, heart disease, chronic kidney disease, liver cirrhosis, and chronic hepatitis). A medical chart review confirmed the answers provided. For those with no history of diabetes, a fasting blood glucose level was obtained. Diabetes was defined according to American Diabetes Association guidelines. For those without a history of hypertension, blood pressure was measured with a mercury sphygmomanometer on the left arm after the patient had been resting for 20 min in a seated position. Hypertension was defined according to the 2017 Hypertension Clinical Practice Guidelines (systolic blood pressure $\geq$ 140 mmHg, diastolic blood pressure $\geq$ 90 mmHg, or the use of antihypertensive medication) [21].

### 2.4. Laboratory Analysis

Venous blood was sampled overnight. Assays for high-sensitivity C-reactive protein were carried out in the Department of Laboratory Medicine, Keelung Chang Gung Memorial Hospital. Biomarkers including IL6, IL8, leptin, adiponectin, and transforming growth factor beta-1 were measured using commercially available enzyme-linked immunosorbent assays (Boster Biological Technology, Pleasanton, CA, USA).

### 2.5. Statistics

Two independent sample *t*-tests were used to compare differences between the continuous variables of the groups, and results were presented as the mean $\pm$ standard deviation (SD). The $\chi2$ test was used to differentiate between the distribution of categorical variables, and results were expressed using frequencies and percentages between the groups. The 3D body surface measurements were screened using a two-sample *t*-test by comparing differences between CAD patients and controls. To avoid collinearity in the regression analysis, one body measurement with the lowest *p* value was selected from each anatomic dimension for subsequent multivariable analysis. A logistic regression model was used to determine the strength of the association between the selected body measurements and the presence of CAD. In addition to the forced-in sociodemographic variables, a backward model selection with *p* < 0.1 was used to determine variables, including lifestyle variables, to be retained in the regression model. The modulating effect was examined by comparing models with and without biomarkers while calculating the strength of association (odds ratio [OR]) between the body measurement combinations and CAD. In order to find associations with CAD in individual patients, biochemical and body shape variables that significantly differed between the non-CAD and CAD groups were further analyzed. This was done by calculating optimal cutoff values for continuous variables using a receiver operating characteristic (ROC) analysis. The statistical software used for the analyses in this study was SPSS 25.0 (IBM Corporation, Armonk, NY, USA).

### 3. Results

A total of 98 patients were recruited into each of the CAD group and control group over a period between March 2016 and December 2017. Baseline characteristics for the study participants are shown in Table 1. Both groups were matched for age and sex, with 76.53% of patients being male and 72.45% equal to or greater than 50 years of age in both CAD and control groups. More patients in the CAD group had a lower educational level (73.47% vs. 37.76%, *p* = 0.001). Among lifestyle variables, the CAD group had more smokers (56.12% vs. 36.73%, *p* = 0.0099)), more patients that did not consume coffee (54.08% vs. 35.71%, *p* = 0.0097), and more that did not exercise (52.04% vs. 37.76%, *p* = 0.044). As regards to risk factors, more patients in the CAD group had hypertension (50% vs. 1.02%,

$p < 0.0001$) and diabetes (36.73% vs. 3.06%, $p < 0.0001$), and fewer patients in the CAD group had hyperlipidemia (20.41% vs. 39.8%, $p = 0.0031$).

**Table 1.** Characteristics of study participants.

| | No CAD | | CAD | | *p* Value |
|---|---|---|---|---|---|
| | n = 98 | | n = 98 | | |
| | Number | % | Number | % | |
| Sex | | | | | 1 |
| Male | 75 | 76.53 | 75 | 76.53 | |
| Female | 23 | 23.47 | 23 | 23.47 | |
| Age (years) | | | | | 0.9971 |
| <50 | 27 | 27.55 | 27 | 27.55 | |
| 50–54 | 18 | 18.37 | 17 | 17.35 | |
| 55–59 | 18 | 18.37 | 17 | 17.35 | |
| 60–64 | 19 | 19.39 | 21 | 21.43 | |
| 65> | 16 | 16.33 | 16 | 16.33 | |
| Occupation | | | | | 0.0954 |
| Government employees | 21 | 21.43 | 12 | 12.24 | |
| Others † | 57 | 58.16 | 71 | 72.45 | |
| Housekeepers/students | 20 | 20.41 | 15 | 15.31 | |
| Educational level | | | | | 0.001 |
| Senior high school | 37 | 37.76 | 72 | 73.47 | |
| College and above | 61 | 62.24 | 26 | 26.53 | |
| Marital status | | | | | 0.3453 |
| Others | 32 | 32.65 | 25 | 25.51 | |
| Married | 66 | 67.35 | 73 | 74.49 | |
| Lifestyle factors | | | | | |
| Tobacco smoking | | | | | 0.0099 |
| No | 62 | 63.27 | 43 | 43.88 | |
| Yes | 36 | 36.73 | 55 | 56.12 | |
| Alcohol consumption | | | | | 1 |
| No | 63 | 64.29 | 64 | 65.31 | |
| Yes | 35 | 35.71 | 34 | 34.69 | |
| Tea consumption | | | | | 0.668 |
| No | 49 | 50 | 45 | 45.92 | |
| Yes | 49 | 50 | 53 | 54.08 | |
| Coffee consumption | | | | | 0.0097 |
| No | 35 | 35.71 | 53 | 54.08 | |
| Yes | 63 | 64.29 | 45 | 45.92 | |
| Betel nut chewing | | | | | 0.1405 |
| No | 89 | 90.82 | 81 | 82.65 | |
| Yes | 9 | 9.18 | 17 | 17.35 | |
| Exercise | | | | | 0.0444 |
| No | 37 | 37.76 | 51 | 52.04 | |
| Yes | 61 | 62.24 | 47 | 47.96 | |
| Diseases | | | | | |
| Hypertension | | | | | <0.0001 |
| No | 97 | 98.98 | 49 | 50 | |
| Yes | 1 | 1.02 | 49 | 50 | |
| Diabetes | | | | | <0.0001 |
| No | 95 | 96.94 | 62 | 63.27 | |
| Yes | 3 | 3.06 | 36 | 36.73 | |
| Hyperlipidemia | | | | | 0.0031 |
| No | 59 | 60.2 | 78 | 79.59 | |
| Yes | 39 | 39.8 | 20 | 20.41 | |

CAD: coronary artery disease. †: Workers, businessmen, freelance workers, or service workers.

Various biomarkers showed some differences between the CAD and control groups. (Table 2) Levels of HsCRP, IL6, IL8, and leptin were higher and adiponectin lower in the CAD group.

**Table 2.** Distribution of biomarkers between study groups.

| | No CAD | | | CAD | | | *p* Value |
|---|---|---|---|---|---|---|---|
| | Means ± std. | | | Means ± std. | | | |
| HsCRP (mg/L) | 1.380 | ± | 3.247 | 3.683 | ± | 7.801 | <0.0001 |
| IL6 (pg/mL) | 5.130 | ± | 4.403 | 10.880 | ± | 22.255 | 0.0141 |
| IL8 (pg/mL) | 10.967 | ± | 6.210 | 15.134 | ± | 11.284 | 0.0018 |
| Leptin (ng/mL) | 3.874 | ± | 2.941 | 9.107 | ± | 13.380 | 0.0003 |
| Adiponectin (μg/mL) | 41.826 | ± | 59.569 | 25.253 | ± | 39.202 | 0.0233 |
| TGFB1 (pg/mL) | 94.880 | ± | 285.200 | 52.659 | ± | 90.076 | 0.1671 |

CAD: coronary artery disease; HsCRP: high-sensitivity C-reactive protein; IL6: interleukin 6; IL8: interleukin 8; TGFB1: transforming growth factor beta-1.

The 3D body surface scanning measurement results are shown in Table 3. The majority of measurements showed significant difference between the two groups, mainly with the CAD group having larger body measurements than controls. Associations between body measurements and biomarkers are presented in Table 4.

**Table 3.** Comparison of body measurements between study groups.

| | No CAD | | CAD | | *p* Value |
|---|---|---|---|---|---|
| | Means ± std. | | Means ± std. | | |
| **Whole body** | | | | | |
| Height (cm) | 167 | ±8.422 | 164.8 | ±7.682 | 0.0577 |
| Weight (kg) | 66.502 | ±12.599 | 72.063 | ±13.134 | 0.0028 |
| Body mass index (kg/m$^2$) | 23.714 | ±3.299 | 26.421 | ±3.720 | <0.0001 |
| Waist hip ratio (WHR) | 0.895 | ±0.059 | 0.941 | ±0.048 | <0.0001 |
| Waist height ratio (WHtR) | 0.52 | ±0.059 | 0.583 | ±0.062 | <0.0001 |
| Waist thigh ratio (WTR) | 2.684 | ±1.261 | 1.853 | ±0.157 | <0.0001 |
| **Head and neck** | | | | | |
| Head surface (cm$^2$) | 1527 | ±125.5 | 1534.7 | ±145.2 | 0.6929 |
| Head volume (cm$^3$) | 5316.4 | ±591.2 | 5484.3 | ±585.0 | 0.0471 |
| Neck circumference (cm) | 41.759 | ±4.214 | 43.252 | ±4.140 | 0.0132 |
| **Trunk** | | | | | |
| Chest width (cm) | 33.03 | ±3.522 | 34.266 | ±3.191 | 0.0108 |
| Chest circumference (cm) | 97.22 | ±8.933 | 103.8 | ±8.262 | <0.0001 |
| Chest sectional area (cm$^2$) | 7020.2 | ±1156.1 | 7585.9 | ±1212.3 | 0.0011 |
| Waist width (cm) | 31.831 | ±3.257 | 33.649 | ±3.184 | 0.0001 |
| Waist circumference (cm) | 87.212 | ±11.060 | 95.92 | ±9.798 | <0.0001 |
| Waist sectional area (cm$^2$) | 6742.6 | ±1247.3 | 7277.4 | ±1572.8 | 0.009 |
| Trunk surface area (cm$^2$) | 7556.9 | ±1120.9 | 7919.1 | ±1180.0 | 0.0287 |
| Trunk volume (cm$^3$) | 45,278.3 | ±9732.4 | 49,391.8 | ±9890.0 | 0.0037 |
| **Hip** | | | | | |
| Hip width (cm) | 35.711 | ±2.814 | 35.685 | ±2.512 | 0.9456 |
| Hip circumference (cm) | 96.699 | ±8.869 | 101.9 | ±8.052 | <0.0001 |
| **Upper limbs** | | | | | |
| Arm length (cm) | | | | | |
| Left | 52.766 | ±3.399 | 50.677 | ±3.904 | <0.0001 |
| Right | 52.917 | ±3.441 | 50.73 | ±3.869 | <0.0001 |

**Table 3.** *Cont.*

| | | No CAD | | CAD | | *p* Value |
|---|---|---|---|---|---|---|
| | | Means ± std. | | Means ± std. | | |
| Upper arm circumference (cm) | | | | | | |
| Left | | 30.331 | ±2.954 | 31.711 | ±3.245 | 0.0021 |
| Right | | 30.383 | ±3.036 | 31.624 | ±3.377 | 0.0074 |
| Forearm circumference (cm) | | | | | | |
| Left | | 21.631 | ±2.778 | 22.885 | ±2.966 | 0.0026 |
| Right | | 21.973 | ±2.884 | 23.065 | ±2.962 | 0.0096 |
| Arm surface area (cm$^2$) | | | | | | |
| Left | | 1204.3 | ±146.2 | 1238.4 | ±146.8 | 0.1042 |
| Right | | 1248.9 | ±151.8 | 1279.3 | ±154.8 | 0.1656 |
| Arm volume (cm$^3$) | | | | | | |
| Left | | 2082.9 | ±354.0 | 2160.1 | ±408.5 | 0.159 |
| Right | | 2098.9 | ±355.2 | 2213 | ±447.8 | 0.0497 |
| Lower limbs | | | | | | |
| Thigh circumference (cm) | | | | | | |
| Left | | 51.811 | ±4.319 | 51.855 | ±4.298 | 0.9438 |
| Right | | 51.852 | ±4.248 | 51.855 | ±4.379 | 0.9963 |
| Leg length (cm) | | | | | | |
| Left | | 70.079 | ±4.532 | 68.516 | ±5.047 | 0.0237 |
| Right | | 70.129 | ±4.448 | 68.441 | ±4.803 | 0.0115 |
| Calf circumference (cm) | | | | | | |
| Left | | 31.533 | ±5.267 | 31.542 | ±4.661 | 0.9907 |
| Right | | 31.265 | ±4.171 | 31.773 | ±4.751 | 0.4271 |
| Knee circumference (cm) | | | | | | |
| Left | | 39.631 | ±3.773 | 40.17 | ±4.313 | 0.3536 |
| Right | | 39.797 | ±3.810 | 40.119 | ±4.473 | 0.5878 |
| Leg surface area (cm$^2$) | | | | | | |
| Left | | 2822.1 | ±546.9 | 2808.2 | ±496.3 | 0.8526 |
| Right | | 2836.3 | ±538.0 | 2822.8 | ±512.9 | 0.8577 |
| Leg volume (cm$^3$) | | | | | | |
| Left | | 6301.1 | ±1382.5 | 6399.2 | ±1444.6 | 0.6276 |
| Right | | 6265.4 | ±1416.0 | 6411.3 | ±1427.4 | 0.4734 |

CAD: coronary artery disease.

**Table 4.** Association between body measurements and biomarkers.

| | | HsCRP (mg/L) | IL6 (pg/mL) | IL8 (pg/mL) | Leptin (ng/mL) | Adiponectin (µg/mL) | TGFB1 (pg/mL) |
|---|---|---|---|---|---|---|---|
| Whole body | | | | | | | |
| Height (cm) | r | −0.13718 | 0.01811 | 0.07323 | −0.16755 | −0.10611 | −0.07586 |
| | *p* value | 0.0552 | 0.8021 | 0.3102 | 0.0195 | 0.1409 | 0.2931 |
| Weight (kg) | r | −0.03163 | 0.0954 | 0.17338 | 0.06635 | −0.24858 | −0.14674 |
| | *p* value | 0.6599 | 0.1858 | 0.0156 | 0.358 | 0.0005 | 0.0412 |
| Body mass index (kg/m$^2$) | r | 0.04459 | 0.11043 | 0.16908 | 0.1982 | −0.24935 | −0.14787 |
| | *p* value | 0.5349 | 0.1253 | 0.0184 | 0.0056 | 0.0005 | 0.0396 |
| Waist hip ratio (WHR) | r | 0.09879 | 0.00763 | 0.09921 | 0.19655 | −0.27043 | −0.20724 |
| | *p* value | 0.1683 | 0.9159 | 0.1687 | 0.006 | 0.0001 | 0.0037 |
| Waist height ratio (WHtR) | r | 0.11197 | 0.09778 | 0.12999 | 0.25614 | −0.16595 | −0.09625 |
| | *p* value | 0.1182 | 0.175 | 0.0708 | 0.0003 | 0.0207 | 0.1819 |
| Waist thigh ratio (WTR) | r | −0.09832 | 0.05043 | 0.05547 | −0.084 | 0.63179 | 0.21122 |
| | *p* value | 0.1704 | 0.485 | 0.4424 | 0.2443 | <0.0001 | 0.0031 |
| Head and neck | | | | | | | |
| Head surface (cm$^2$) | r | −0.08494 | 0.0846 | 0.19626 | 0.13819 | 0.15984 | −0.01762 |
| | *p* value | 0.2366 | 0.2408 | 0.0061 | 0.0547 | 0.026 | 0.8074 |

**Table 4.** *Cont.*

| | | HsCRP (mg/L) | IL6 (pg/mL) | IL8 (pg/mL) | Leptin (ng/mL) | Adiponectin (µg/mL) | TGFB1 (pg/mL) |
|---|---|---|---|---|---|---|---|
| Head volume (cm$^3$) | r | −0.10987 | 0.1272 | 0.17475 | 0.24607 | 0.14232 | −0.03866 |
| | *p* value | 0.1253 | 0.0772 | 0.0148 | 0.0005 | 0.0477 | 0.5925 |
| Neck circumference (cm) | r | 0.02111 | 0.03145 | 0.13184 | −0.00705 | −0.21413 | −0.10159 |
| | *p* value | 0.769 | 0.6633 | 0.0669 | 0.9223 | 0.0027 | 0.1587 |
| Trunk | | | | | | | |
| Chest width (cm) | r | 0.0262 | 0.10839 | 0.21173 | 0.08792 | −0.10738 | −0.12126 |
| | *p* value | 0.7154 | 0.1325 | 0.003 | 0.2228 | 0.1362 | 0.0921 |
| Chest circumference (cm) | r | 0.0235 | 0.11379 | 0.19721 | 0.18524 | −0.13333 | −0.16592 |
| | *p* value | 0.7437 | 0.1141 | 0.0058 | 0.0097 | 0.0638 | 0.0208 |
| Chest sectional area (cm$^2$) | r | −0.01108 | 0.09529 | 0.18221 | 0.08779 | −0.12291 | −0.07173 |
| | *p* value | 0.8775 | 0.1863 | 0.011 | 0.2235 | 0.0878 | 0.3203 |
| Waist width (cm) | r | 0.05531 | 0.08967 | 0.19375 | 0.13998 | −0.14666 | −0.17349 |
| | *p* value | 0.4413 | 0.2137 | 0.0068 | 0.0516 | 0.0413 | 0.0156 |
| Waist circumference (cm) | r | 0.05435 | 0.09742 | 0.1501 | 0.18334 | −0.21226 | −0.11769 |
| | *p* value | 0.4493 | 0.1766 | 0.0367 | 0.0105 | 0.003 | 0.1022 |
| Waist sectional area (cm$^2$) | r | 0.00772 | 0.07819 | 0.19018 | 0.12382 | −0.05133 | −0.07408 |
| | *p* value | 0.9145 | 0.2785 | 0.0079 | 0.0854 | 0.4772 | 0.3047 |
| Truck surface area (cm$^2$) | r | 0.00952 | 0.03845 | 0.14888 | 0.09488 | −0.02907 | −0.07203 |
| | *p* value | 0.8947 | 0.5945 | 0.0383 | 0.1882 | 0.6874 | 0.3183 |
| Truck volume (cm$^3$) | r | −0.05931 | 0.0808 | 0.15553 | 0.07081 | −0.21734 | −0.09545 |
| | *p* value | 0.4089 | 0.2627 | 0.0304 | 0.3265 | 0.0023 | 0.1855 |
| Hip | | | | | | | |
| Hip width (cm) | r | −0.055 | 0.01265 | 0.08126 | 0.03089 | 0.0362 | −0.10802 |
| | *p* value | 0.4439 | 0.8611 | 0.26 | 0.669 | 0.6163 | 0.1338 |
| Hip circumference (cm) | r | 0.00261 | 0.14663 | 0.1686 | 0.11914 | −0.08771 | −0.02172 |
| | *p* value | 0.971 | 0.0413 | 0.0188 | 0.098 | 0.2239 | 0.7637 |
| Upper limbs Arm length (cm) | | | | | | | |
| Left | r | −0.07267 | −0.11303 | −0.13079 | −0.23207 | −0.14424 | −0.0883 |
| | *p* value | 0.3114 | 0.1166 | 0.0691 | 0.0011 | 0.0448 | 0.2208 |
| Right | r | −0.06768 | −0.12305 | −0.13273 | −0.23011 | −0.13388 | −0.08655 |
| | *p* value | 0.3459 | 0.0874 | 0.065 | 0.0012 | 0.0627 | 0.2301 |
| Upper arm circumference (cm) | | | | | | | |
| Left | r | 0.01184 | 0.05659 | 0.15481 | 0.14534 | −0.09839 | −0.12129 |
| | *p* value | 0.8692 | 0.4332 | 0.0311 | 0.0432 | 0.1723 | 0.0921 |
| Right | r | −0.00758 | 0.03127 | 0.16224 | 0.14506 | −0.13694 | −0.11651 |
| | *p* value | 0.916 | 0.6651 | 0.0238 | 0.0436 | 0.0569 | 0.1057 |
| Forearm circumference (cm$^2$) | | | | | | | |
| Left | r | −0.04892 | 0.11434 | 0.17707 | 0.03087 | −0.33442 | −0.16539 |
| | *p* value | 0.4959 | 0.1124 | 0.0135 | 0.6691 | <0.0001 | 0.0212 |
| Right | r | −0.06589 | 0.06946 | 0.16128 | 0.01246 | −0.36135 | −0.16901 |
| | *p* value | 0.3589 | 0.3358 | 0.0247 | 0.8631 | <0.0001 | 0.0185 |
| Arm surface area (cm$^2$) | | | | | | | |
| Left | r | 0.09348 | 0.05002 | 0.01059 | −0.03262 | −0.37273 | −0.19753 |
| | *p* value | 0.1925 | 0.4885 | 0.8835 | 0.6516 | <0.0001 | 0.0058 |
| Right | r | 0.02712 | 0.00412 | 0.07986 | −0.0347 | −0.3536 | −0.17611 |
| | *p* value | 0.706 | 0.9546 | 0.2683 | 0.631 | <0.0001 | 0.014 |
| Arm volume (cm$^3$) | | | | | | | |
| Left | r | −0.01615 | 0.06738 | 0.01326 | 0.05721 | −0.18919 | −0.11855 |
| | *p* value | 0.8222 | 0.3506 | 0.8544 | 0.4282 | 0.0082 | 0.0997 |
| Right | r | −0.03485 | 0.06145 | 0.13408 | 0.04729 | −0.21427 | −0.10349 |
| | *p* value | 0.6278 | 0.3947 | 0.0623 | 0.5126 | 0.0027 | 0.151 |
| Lower limbs Thigh circumference (cm) | | | | | | | |
| Left | r | −0.05921 | 0.05597 | 0.16145 | 0.11974 | 0.1302 | −0.04956 |
| | *p* value | 0.4097 | 0.4383 | 0.0245 | 0.0963 | 0.0704 | 0.4925 |
| Right | r | −0.05103 | 0.04722 | 0.16389 | 0.11624 | 0.16344 | −0.04332 |
| | *p* value | 0.4775 | 0.5132 | 0.0224 | 0.1065 | 0.0228 | 0.5487 |

**Table 4.** *Cont.*

|  |  | HsCRP (mg/L) | IL6 (pg/mL) | IL8 (pg/mL) | Leptin (ng/mL) | Adiponectin (µg/mL) | TGFB1 (pg/mL) |
|---|---|---|---|---|---|---|---|
| Leg length (cm) |  |  |  |  |  |  |  |
| Left | r | 0.00066 | 0.02189 | 0.02015 | −0.12793 | −0.09456 | −0.07592 |
|  | *p* value | 0.9927 | 0.7619 | 0.7804 | 0.0755 | 0.1897 | 0.2928 |
| Right | r | 0.00283 | 0.01332 | 0.01618 | −0.1234 | −0.08464 | −0.06908 |
|  | *p* value | 0.9686 | 0.8538 | 0.8228 | 0.0865 | 0.2406 | 0.3385 |
| Calf circumference (cm) |  |  |  |  |  |  |  |
| Left | r | −0.1081 | 0.09277 | 0.14462 | 0.02851 | −0.0768 | −0.04167 |
|  | *p* value | 0.1315 | 0.1982 | 0.0442 | 0.6931 | 0.2871 | 0.564 |
| Right | r | −0.10161 | 0.09043 | 0.1495 | 0.05331 | −0.04868 | −0.04637 |
|  | *p* value | 0.1565 | 0.2099 | 0.0375 | 0.4603 | 0.5003 | 0.5209 |
| Knee circumference (cm) |  |  |  |  |  |  |  |
| Left | r | −0.02025 | 0.0434 | 0.09408 | 0.22329 | 0.13504 | −0.12404 |
|  | *p* value | 0.7781 | 0.548 | 0.192 | 0.0018 | 0.0605 | 0.0848 |
| Right | r | −0.06809 | 0.02673 | 0.09492 | 0.21495 | 0.13828 | −0.11538 |
|  | *p* value | 0.343 | 0.7115 | 0.188 | 0.0026 | 0.0545 | 0.1092 |
| Leg surface area (cm$^2$) |  |  |  |  |  |  |  |
| Left | r | −0.04733 | −0.00004 | 0.09206 | 0.19307 | 0.23322 | −0.06176 |
|  | *p* value | 0.5101 | 0.9996 | 0.2017 | 0.007 | 0.0011 | 0.3923 |
| Right | r | −0.05113 | −0.01193 | 0.0916 | 0.20009 | 0.24986 | −0.04194 |
|  | *p* value | 0.4767 | 0.8689 | 0.204 | 0.0052 | 0.0004 | 0.5615 |
| Leg volume (cm$^3$) |  |  |  |  |  |  |  |
| Left | r | −0.05158 | 0.03393 | 0.10165 | 0.19486 | 0.13742 | −0.06532 |
|  | *p* value | 0.4728 | 0.6385 | 0.1584 | 0.0065 | 0.056 | 0.3655 |
| Right | r | −0.04851 | 0.02795 | 0.08977 | 0.2046 | 0.14678 | −0.06536 |
|  | *p* value | 0.4996 | 0.6989 | 0.2132 | 0.0042 | 0.0411 | 0.3652 |

*Multiple Logistic Regression and ROC Analysis*

The associations between different body measurements and biomarkers on the occurrence of CAD were further assessed using a multiple logistic regression model adjusted for sex, age, education, exercise, smoking, alcohol drinking and coffee consumption, hypertension, diabetes, and hyperlipidemia. It was found that CW (OR 0.761, 95% CI = 0.586–0.987, *p* = 0.0399), RAL (OR 0.743, 95% CI = 0.632–0.875, *p* = 0.0004), WC (OR 1.119, 95% CI = 1.035–1.21, *p* = 0.0048), leptin (OR 1.443, 95% CI = 1.184–1.76, *p* = 0.0003), adiponectin (OR 0.978, 95% CI = 0.963–0.994, *p* = 0.006), and IL6 (OR 1.181, 95% CI = 1.021–1.366, *p* = 0.0254 were significantly associated with CAD (Table 5).

The biomarker score, body measurement score, biomarker and body measurement score ware calculated based on the estimated values generated by the Table 5 model, and the scores were adjusted by risk factors. Receiver operating characteristic (ROC) curve analyses were adopted to estimate the predictive values of biomarker score, body measurement score and biomarkers combined with body measurements score for the occurrence of CAD. It was found that the combination of biomarker scores and body measurement scores had the greatest area under the curve and best association with CAD as shown in Figure 1 and Table 6. (Area under the curve of 0.8056, 95% CI = 0.7450–0.8662, *p* < 0.0001).

Table 5. Multiple logistic regression analysis of developing CAD.

| | Biomarkers | | | | Body Measurements | | | | Biomarkers and Body Measurements | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Multivariable Logistic Regression Analysis † | | | | Multivariable Logistic Regression Analysis † | | | | Multivariable Logistic Regression Analysis † | | | |
| | ORs | Lower | Upper | p Value | ORs | Lower | Upper | p Value | ORs | Lower | Upper | p Value |
| Chest width (cm) | | | | | 0.761 | 0.586 | 0.987 | 0.0399 | 0.62 | 0.441 | 0.872 | 0.0061 |
| Right arm length (cm) | | | | | 0.743 | 0.632 | 0.875 | 0.0004 | 0.738 | 0.603 | 0.904 | 0.0033 |
| Waist circumference (cm) | | | | | 1.119 | 1.035 | 1.21 | 0.0048 | 1.15 | 1.038 | 1.275 | 0.0078 |
| Leptin (ng/mL) | 1.443 | 1.184 | 1.76 | 0.0003 | | | | | 1.504 | 1.197 | 1.89 | 0.0005 |
| Adiponectin (µg/mL) | 0.978 | 0.963 | 0.994 | 0.006 | | | | | 0.976 | 0.959 | 0.994 | 0.0094 |
| IL6 (pg/mL) | 1.181 | 1.021 | 1.366 | 0.0254 | | | | | 1.256 | 1.033 | 1.528 | 0.0224 |

CAD: coronary artery disease. †: Adjusted for sex, age, education, exercise, smoking, alcohol and coffee intake, hypertension, diabetes, and hyperlipidemia.
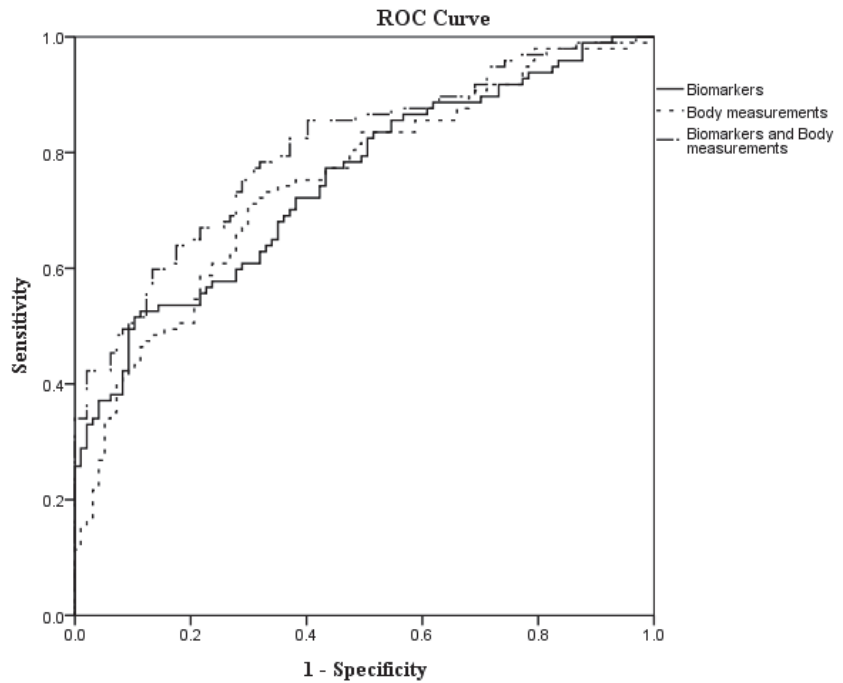
**Figure 1.** The receiver operating characteristic (ROC) curves to predict CAD.

**Table 6.** Area under the receiver operating characteristic curve (AUROC) and 95% confidence interval (CI) for the different scores.

|  | AUROC | 95%CI | | *p* Value |
|---|---|---|---|---|
| Biomarkers score † | 0.7492 | (0.6812 | 0.8172) | <0.0001 |
| Body measurements score † | 0.7476 | (0.6791 | 0.8161) | <0.0001 |
| Biomarkers and body measurements score † | 0.8056 | (0.7450 | 0.8662) | <0.0001 |
| Biomarkers score = (−1.9727) + 0.3669 × Leptin + (−0.0218) × Adiponectin + 0.1661 × IL6 | | | | |
| Body measurements score = 13.0994 + (−0.2735) × CW + 0.1125 × WC + (−0.2967) × RAL | | | | |
| Biomarkers and body measurements score = (15.3447) + 0.4082 × Leptin + (−0.0239) × Adiponectin + 0.2282 × IL6 + (−0.4781) × CW + 0.14 × WC + (−0.3034) × RAL | | | | |

† Adjusted for sex, age, education, exercise, smoking, alcohol and coffee intake, hypertension, diabetes and hyperlipidemia.

## 4. Discussion

Our study results show that lower educational level, no coffee consumption, physical inactivity, low adiponectin, high leptin, and high IL6 levels were associated with CAD. In terms of 3D body measurements, compared to the traditional BMI assessment, smaller CW and RAL with higher WC were also associated with CAD. In addition, the combination of biomarker scores and body measurement scores had the highest predictive value for CAD as shown with ROC analysis. These findings have given us a novel method for assessing the risk of those who may have CAD.

Inflammation contributes to CAD, among which IL6 plays an important role in atherogenesis and atherosclerotic plaque destabilization. IL6 is associated with vascular endothelial injury and tissue fibrosis, promotes angiogenesis, and increases vascular permeability [22]. Once IL6 levels are abnormally elevated, a series of pathological changes occurs including inflammatory injury, plaque formation and rupture, and thrombosis. Chronic

exposure to IL6 also disturbs insulin action and body fat. Yet, despite having proinflammatory properties, IL6 also plays an important role in anti-inflammation. Enhanced fat oxidation occurs when IL6 is increased acutely, leading to improved insulin-stimulated glucose uptake with anti-inflammatory effects. With chronic secretion under obese conditions, these effects are not seen, probably due to the development of IL6 resistance [23]. In our study, it was found that IL6 was associated with CAD.

Adipose tissue is associated with CAD, abdominal adiposity causes development of adipose cells that are enlarged and dysfunctional [24]. These dysfunctional adipose tissues secrete pro-inflammatory biomarkers including prostaglandins, C-reactive protein, and cytokines such as interleukins and leptin with a decrease in adiponectin levels [25,26]. Leptin can cause vascular smooth muscle hypertrophy and oxidative stress, and stimulates vascular inflammation which may then lead to the development of type 2 diabetes mellitus, hypertension, atherosclerosis, and CAD [27]. Some studies have shown that increased leptin levels in plasma are associated with adverse outcomes in heart failure and CAD [28]. In CAD patients, higher serum leptin levels were significantly related to an increasing number of stenotic coronary arteries and arterial stiffness [29]. Another adipokine, adiponectin, also has important effects on the cardiovascular system. Its levels are negatively correlated with metabolic and cardiovascular disorders [30], with low levels having been shown to be an independent risk factor for cardiovascular disease [31,32]. In contrast to leptin, adiponectin levels are directly correlated with insulin sensitivity and inversely correlated with adiposity [33–35]. Certainly, as shown in our study population, the above mentioned adipokines were found to be associated with CAD.

By using a more accurate 3D body scanning method, we found that higher WC, lower CW and lower RAL were also associated with CAD. WC, which reflects abdominal obesity, has been suggested to be superior to BMI for CAD risk prediction [36], and this was similarly seen in our study. In addition to the important role it has in CAD, leptin has also been found to affect bone metabolism via both direct and indirect mechanisms [37]. Studies have shown that leptin resistance or insulin resistance as found in obesity may lead to poorer bone health [38,39]. Increased adiposity can also lead to decreased bone mass, affecting cortical bone more than trabecular bone [40,41]. These mechanisms may help explain the findings of shorter RAL associated with CAD in our study. Interestingly, CW was associated with CAD in our population. The thoracic cavity, when intact and closed, constrains the heart and lungs to a limited space, such that intrathoracic pressure changes throughout respiratory phases can have varying effects on cardiac function. Thus, one with a smaller chest width may have impaired pulmonary function or motion capacity of organs in the chest in addition to limitation of circulation flow rates. It has been shown that small whole heart volume predicts cardiovascular events in patients with stable chest pain [42]. During normal breathing, chest wall motion is determined by the displacement from respiration and the displacement by heart activity. There has been an interest in how chest wall motion provides information on the cardiorespiratory system with the design of different chest wall models [43]. A smaller CW may therefore also be an indicator that there is restricted cardiopulmonary displacement from cardiovascular impairment. Dynamic lung and chest wall compliance can be measured by the pressure–volume curve [44]. In fact, it has been documented that abdominal obesity preferentially depresses chest wall compliance resulting in a marked decrease in functional residual capacity and expiratory reserve volume [45]. This may very well explain the link between the high WC and lower CW we see associated with CAD in our population.

Faced with CAD being such an important cause of death worldwide, we sought to explore its associations with the more accurate method of 3D-derived body measurements and biomarkers in an attempt to gain more mechanistic insight.

*Limitations*

As our study design was of a cross-sectional study, we are unable to infer causality from the results. The 3D body measurements were performed only at one point in time

with no repeated estimations or data on changes over time. Our study population was of Chinese adults in a hospital setting so the results might not be applied to other ethnicities, age groups, or populations in the community. Therefore, it may be necessary to clarify these conclusions in further longitudinal studies and in a wider population.

## 5. Conclusions

In our study, 3D anthropometrics provide incremental information regarding associations of body surface measurements with CAD. It has been shown that shorter RAL and CW, and longer WC measurements combined with lower adiponectin and higher leptin and IL6 levels were associated with CAD. Although the precise mechanisms are far from clear, by combining non-invasive 3D body surface measurements together with biomarkers, we may in future be able to explore a different mechanistic approach to CAD, and non-invasively identify those with this condition in clinical practice, in addition to providing more information in epidemiological studies and preventative medicine.

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# Effectiveness and Safety of Dabigatran Reversal with Idarucizumab in the Taiwanese Population: A Comparison Based on Eligibility for Inclusion in Clinical Trials

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**Abstract:** *Background and Objectives:* The effectiveness and safety of idarucizumab for the reversal of the effects of dabigatran have been proven. However, there remains a paucity of literature comprehensively investigating outcomes in real-world patients. This is especially true when comparing patients who were eligible for inclusion in the RE-VERSE AD trial with patients who were ineligible. As the prescription of dabigatran has become increasingly popular, the generalizability of the results to real-world populations has come into question due to the broad variability of real-world patients receiving dabigatran. Our study aimed to identify all patients who were prescribed idarucizumab and examined how effectiveness and safety varied among those patients who were eligible and ineligible for the trial. *Materials and Methods*: This retrospective cohort study analyzed the largest medical database in Taiwan. We enrolled all patients who were prescribed and received idarucizumab from when it became available in Taiwan up until May 2021. A Total of 32 patients were included and analyzed, and they were further divided into subgroups based on their eligibility for inclusion in the RE-VERSE AD trial. Multiple outcomes were evaluated, including successful hemostasis rate, complete reversal efficacy of idarucizumab, 90-day thromboembolic events, intra-hospital mortality, and adverse event rate. *Results*: In our study, we found that 34.4% of real-world cases of idarucizumab use were ineligible for the RE-VERSE AD trials. The eligible group had higher successful hemostasis rates (95.2% vs. 80%) and anticoagulant effect reversal rates compared to the ineligible group (73.3% vs. 0%). The mortality rates were 9.5%, compared to 27.3% in the ineligible group. Few adverse effects (n = 3) and 90-day thromboembolic events (n = 1) were observed in either group. Among the ineligible cases, all acute ischemic stroke patients (n = 5) received definite, timely treatments without complications. *Conclusions:* Our study demonstrated the real-world effectiveness and safety of idarucizumab infusion for trial-eligible patients and all acute ischemic stroke patients. However, although it seems to be effective and safe, idarucizumab appears to be less effective in other trial-ineligible patients. Despite this result, our study provides further evidence for extending the applicability of idarucizumab in real-world scenarios. Our study suggests that idarucizumab can be a safe and effective option for reversing the anticoagulant effect of dabigatran, particularly for eligible patients.

**Keywords:** idarucizumab; dabigatran; reversal agents

## 1. Introduction

Dabigatran is widely used due to its clinical advantages over other anticoagulants, including its good tolerance, low potential for drug–drug interaction, predictable pharmacokinetics, and absence of need for frequent coagulation monitoring [1–8]. As the

prescription of dabigatran has become gradually more popular, the reported annual major bleeding rate has varied from 2.71 to 3.36% [1]. To manage life-threatening situations associated with dabigatran, idarucizumab, a humanized dabigatran-specific monoclonal antibody with high affinity and specificity, was approved by the U.S. Food and Drug Administration and the European Medicines Agency in 2015 for patients suffering from uncontrolled bleeding or requiring emergency interventions. There are no contraindications for the administration of idarucizumab. [9,10]. The RE-VERSE AD study showed that idarucizumab is a rapid, safe, and lasting reversal agent in life-threatening scenarios, with an average hemostasis rate of 80.4% and 13.1% mortality, along with a 4.8% 30-day thromboembolic event rate [11]. The RE-VECTO study, a global surveillance program, further illustrated the global idarucizumab usage pattern in clinical practice and also demonstrated a low percentage of off-label use (<2%) [12].

After initial marketing, the effectiveness and safety of idarucizumab were evaluated through several studies, and these studies demonstrated a high rate of successful hemostasis with only a few thrombotic events or other serious adverse drug events (ADEs) [13–18]. The most commonly reported adverse reactions are headache and erythema [4,9]. However, since the guidance for the use of idarucizumab is based on indications from the preceding trials, the generalizability of idarucizumab has come into question due to the great variability of real-world patients receiving dabigatran [11,19]. Recently, a growing number of scenarios have seemed to benefit from idarucizumab use, such as ischemic stroke patients facing intravenous thrombolysis or intravenous tissue plasminogen activator treatment, which have been widely investigated [20–26]. Therefore, a better understanding of the breadth of applicability of idarucizumab is urgently needed to enhance the safety and well-being of dabigatran-treated patients.

There remains a paucity of comprehensive literature examining outcomes in real-world patients, especially in terms of comparing patients who would have been eligible for inclusion in the RE-VERSE AD trial with those who would have been ineligible. In our study, we aimed to examine all patients who were prescribed idarucizumab in one of the largest medical centers in Taiwan. Our goals were to determine how effectiveness and safety varied between patients who would have met the inclusion criteria of the RE-VERSE AD trial and those who would not have.

## 2. Methods

### 2.1. Study Design and Setting

We performed a retrospective and observational cohort study by analyzing the electronic medical records from the Chang Gung Research Database (CGRD). As one of the largest healthcare providers in Taiwan, Chang Gung Memorial Hospital annually handles an average of 8.6 million outpatient visits and around 370,000 admissions. The CGRD is the largest multi-institutional database in Taiwan, containing individual data from about 6% of the Taiwanese population [27,28]. This study was approved by the Chang Gung Medical Foundation Institutional Review Board, which waived the need for informed consent (IRB Number: 202101259B0).

### 2.2. Study Population, Eligibilities, and the Infusion Protocol for Idarucizumab

The inclusion criteria of our study were being more than 18 years old and receiving dabigatran. The only exclusion criterion was if patients did not actually receive the infusion following prescription. We enrolled all patients who were prescribed idarucizumab from when it became available in Taiwan, up until May 2021. Based on the inclusion criteria for the RE-VERSE AD trial, which can be retrieved from the study protocol of the trials, the potential trial eligibility of the individual patients who received idarucizumab was evaluated. This evaluation of eligibility was conducted by two independent reviewers (C.-H.W., J.-W.D.), whereby disagreements between the two reviewers were resolved in consultation with the senior author (S.-C.L.). We further divided all patients administered idarucizumab into three subgroups based on their eligibility category: Group A (uncontrol-

lable or life-threatening bleeding, which is eligible for the RE-VERSE AD trials), Group B (emergent surgery or invasive procedures, which is eligible for the RE-VERSE AD trials), and Group C (ineligible for the RE-VERSE AD trials). As demonstrated in the RE-VERSE AD trial, adult patients who were receiving dabigatran and required surgery or an invasive procedure that could not be delayed for at least eight hours, or who were experiencing uncontrollable or life-threatening bleeding, were included. Patients who did not receive dabigatran, had minor bleeding, had elective surgery, or had a low risk of uncontrolled bleeding during the procedure were excluded. Following the inclusion and exclusion criteria of the RE-VERSE AD trial, we considered patients ineligible and classified them as Group C if they did not receive dabigatran or did not meet the specific or emergent conditions outlined in the trial. The definition of major and life-threatening bleeding was in accordance with the bleeding scale of the International Society of Thrombosis and Hemostasis (ISTH) [29]. Emergent surgery and invasive procedures were defined as interventions that could not be delayed by more than 8 h and situations where normal hemostasis was required [11].

The suitability of patients for idarucizumab infusion was evaluated by two physicians acting independently. Clinically, idarucizumab is administered intravenously as two consecutive infusions at 2.5 g/50 mL each, with at least a 10 min interval between each infusion.

### 2.3. Data Collection and Outcomes

We retrospectively collected the baseline characteristics for all patients prior to idarucizumab treatment. These parameters included age, sex, body weight, underlying comorbidity such as hypertension, diabetes mellitus, heart failure, previous ischemic stroke, acute coronary syndrome, and previous systemic embolism. We also recorded indications for dabigatran use, the daily dabigatran dosage, and laboratory data both before and after idarucizumab infusion, including creatinine clearance, activated partial thromboplastin time, international normalized ratio, hemoglobin level, and platelet count. Additionally, we calculated the $CHA_2DS_2$-VASc score, HAS-BLED score, and NIHSS score. To retrieve detailed information and the laboratory data of the selected patients, we retrieved electronic medical records from the Chang Gung Research Database (CGRD).

The primary safety outcome was defined as 90-day thromboembolic events, which comprised arterial (i.e., ischemic stroke, myocardial infarction, or peripheral vascular disease) or venous thromboembolism (i.e., deep vein thrombosis or pulmonary embolism). The secondary safety outcome was intra-hospital mortality, which was defined as death by any cause, documented in the medical record after idarucizumab infusion, during hospitalization. The third safety outcome was the rate of adverse events within 30 days. The adverse events included all adverse symptoms that were judged by the investigators to be related to idarucizumab.

The primary effectiveness outcome was the hemostasis rate, which was assessed and defined in accordance with the effectiveness of hemostasis, followed the International Society on Thrombosis and Hemostasis (ISTH) guidance, and varied depending on the situation [30]. The time of infusion of idarucizumab and the day of occurrence of thromboembolic events were both collected. The secondary effectiveness outcome was the reversal efficacy of idarucizumab. Complete reversal of anticoagulant effects was defined as the normalization of the activated partial-thromboplastin time (aPTT) after idarucizumab infusion. We chose aPTT over the diluted thrombin time (dTT) or the ecarin clotting time (ECT), as used in the RE-VERSE AD study, because the latter are not commonly used or available in the usual clinical settings in Taiwan [11]. Thus, we collected the blood sampling time and an aPTT value before and after infusion of idarucizumab. However, it is important to note that normal aPTT might not exclude on-therapy levels of dabigatran, and results should be interpreted cautiously [31].

### 2.4. Statistical Analysis

Descriptive statistics were used in this study. The categorical variables of the baseline demographic are presented in percentages (%) and the continuous variables are expressed as mean ± standard deviation (SD).

### 3. Results

#### 3.1. Characteristics and Eligibility of Patients

We identified 47 patients from the Chang Gung Research Database (CGRD), all of whom were prescribed idarucizumab following its approval for use in Taiwan. However, 15 of these patients eventually did not receive idarucizumab. Among these 15 patients, 12 patients received catheter ablation for atrial fibrillation or left atrial appendage occlusion. Given the risk of bleeding during the procedure, which includes transseptal puncture and ablation for pulmonary vein isolation, operators usually prescribe preparations in case complications arise. One patient was misprescribed and did not receive an infusion, and one was using edoxaban and thus there was no administration of idarucizumab. The other patient was not infused with iarucizumab because no drug was available at that time. The study included the remaining 32 patients who received idarucizumab, with a mean age of 76.2 years and 46.9% being male. All patients received two consecutive infusions of 2.5 g/50 mL each (5 g of Idarucizumab). Of these 32 patients, 21 would have been eligible for the RE-VERSE AD trials. Their mean age was 79.6 years and 47.6% were male. More detailed baseline characteristics of the included patients are listed in Table 1.

**Table 1.** The demographics and clinical data of patients who received idarucizumab.

| Variable | All n = 32 | Eligible for Trials n = 21 | Group A n = 16 | Group B n = 5 | Group C n = 11 | RE-VERSE AD, n = 503 |
|---|---|---|---|---|---|---|
| Male, n (%) | 15 (46.9%) | 10 (47.6%) | 8 (50%) | 2 (40%) | 5 (45.5%) | 274 (54.5%) |
| Age, mean (SD, years old) | 76.2 (11.5) | 79.6 (9.6) | 81.2 (9.3) | 74.6 (9.8) | 69.5 (12.5) | 78 |
| Body weight, median (SD, kg) | 63.6 (14.2) | 60.2 (11.9) | 60.6 (11.8) | 58.9 (13.8) | 70.3 (16.6) | 75 |
| Comorbidity, n (%) | | | | | | |
| Hypertension | 19 (59.3%) | 11 (52.4%) | 10 (62.5%) | 1 (20%) | 8 (72.7%) | 394 (78.3%) |
| Diabetes mellitus | 10 (31.3%) | 8 (38.1%) | 5 (31.3%) | 3 (60%) | 2 (18.2%) | 152 (30.2%) |
| Heart failure | 11 (34.4%) | 8 (38.1%) | 6 (37.5%) | 2 (40%) | 3 (27.3%) | 182 (36.2%) |
| Previous ischemic stroke | 14 (43.8%) | 8 (38.1%) | 5 (31.3%) | 3 (60%) | 6 (54.6%) | 47 (9.3%) |
| Acute coronary syndrome | 3 (9.4%) | 3 (14.3%) | 2 (12.5%) | 1 (20%) | 0 (0%) | 178 (35.4%) |
| Previous systemic embolism | 6 (18.8%) | 5 (23.8%) | 5 (31.3%) | 0 (0%) | 1 (9.1%) | 36 (7.2%) |
| Creatinine clearance, mL/min (%) | | | | | | |
| ≥80 | 9 (28.1%) | 4 (19.0%) | 3 (18.8%) | 1 (20%) | 5 (45.5%) | 108 (21.5%) |
| 30–80 | 20 (62.5%) | 14 (66.7%) | 10 (62.5%) | 4 (80%) | 6 (54.6%) | 290 (57.6%) |
| <30 | 3 (9.4%) | 3 (14.3%) | 3 (18.8%) | 0 (0%) | 0 (0%) | 91 (18.1%) |
| Dabigatran indications | | | | | | |
| Atrial fibrillation | 27 (84.4%) | 19 (90.5%) | 14 (87.5%) | 5 (100%) | 8 (72.7%) | 478 (95%) |
| Systemic embolism | 5 (15.6%) | 4 (19.0%) | 4 (25%) | 0 (0%) | 1 (9.1%) | 9 (1.8) |
| $CHA_2DS_2$-VASc score, median | 4.9 | 5.2 | 5.3 | 5.0 | 4.2 | N/A |
| HAS-BLED score, median | 2.7 | 2.9 | 3.2 | 1.8 | 2.5 | N/A |
| Initial NIHSS score, median | 13.5 | 15 | N/A | 15 | 13.2 | N/A |
| Daily dose of dabigatran, n (%) | | | | | | |
| 150 mg twice daily | 6 (18.8%) | 5 (23.8%) | 4 (25%) | 1 (20%) | 1/7 (14.3%) | 151 (30%) |
| 110 mg twice daily | 22 (68.8%) | 16 (76.2%) | 12 (75%) | 4 (80%) | 6/7 (85.7%) | 311 (61.8%) |
| Successful hemostasis, n (%) | 24/26 (92.3%) | 20/21 (95.2%) | 15 (93.8%) | 5 (100%) | 4/5 (80%) | 80.4% |
| Complete reversal of anticoagulant effects, n (%) [a] | 11/25 (44.0%) | 11/15 (73.3%) | 9/12 (75%) | 2/3 (66.7%) | 0/10 (0%) | N/A |
| Mortality, n (%) [b] | 5 (15.6%) | 2 (9.5%) | 2 (12.5%) | 0 (0%) | 3 (27.3%) | 13.1% |
| Thromboembolic events, n (%) [c] | 1 (3.1%) | 1 (4.8%) | 0 (0%) | 1 (20%) | 0 (0%) | 24 (4.8%) |

**Table 1.** *Cont.*

| Variable | All n = 32 | Eligible for Trials n = 21 | Group A n = 16 | Group B n = 5 | Group C n = 11 | RE-VERSE AD, n = 503 |
|---|---|---|---|---|---|---|
| Rebleeding rate, n (%) | 1/26 (3.9%) | 1/21 (4.8%) | 1 (6.3%) | 0 (0%) | 0/5 (0%) | 10 (2.0%) |
| Resumption of DOAC, n (%) | 20 (62.5%) | 14 (66.7%) | 10 (62.5%) | 4 (80%) | 6 (54.5%) | N/A |
| Choice of DOAC after resumption, n (%) | | | | | | N/A |
| Dabigatran | 9 (28.1%) | 5 (23.8%) | 3 (18.8%) | 2 (40%) | 4 (36.4%) | N/A |
| Apixaban | 4 (12.5%) | 3 (14.3%) | 2 (12.5%) | 1 (20%) | 1 (9.1%) | N/A |
| Edoxaban | 2 (6.3%) | 2 (9.5%) | 2 (12.5%) | 0 (0%) | 0 (0%) | N/A |
| Rivaroxaban | 2 (6.3%) | 1 (4.8%) | 1 (6.3%) | 0 (0%) | 1 (9.1%) | N/A |
| Other anticoagulants/antiplatelets | 3 (9.4%) | 3 (14.3%) | 2 (12.5%) | 1 (20%) | 0 (0%) | N/A |
| Adverse side effects [d], n (%) | 3 (9.4%) | 2 (9.5%) | 0 (0%) | 2 (40%) | 1 (9.1%) | 117 (23.3%) |
| Laboratory data before idarucizumab | | | | | | |
| aPTT, median (s) | 43.6 | 42.3 | 52.9 | 33.7 | 35.7 | N/A |
| Prolonged aPTT, n (%) | 17 (53.1%) | 16 (76.2%) | 13 (81.3%) | 3 (60%) | 1 (9.1%) | 372 (74.2%) |
| INR, median, median (s) | 2.1 | 2.3 | 2.6 | 1.2 | 1.8 | N/A |
| Platelet count, median (1000/µL) | 194.9 | 205.0 | 187.3 | 261.6 | 175.7 | N/A |
| Hemoglobin, median (g/dL) | 11.2 | 10.2 | 9.2 | 13.4 | 13.2 | N/A |
| Laboratory data after idarucizumab | | | | | | |
| aPTT, median (s) | 34.5 | 33.1 | 35 | 25.6 | 38.6 | N/A |
| INR, median, median (s) | 1.3 | 1.3 | 1.3 | 1.1 | 1.3 | N/A |

DOAC: direct oral anticoagulant; aPTT: activated partial thromboplastin time; INR: international normalized ratio. [a] Defined as normalization of the activated partial-thromboplastin time (aPTT) after idarucizumab infusion. [b] Defined as intra-hospital mortality, consisting of death by any cause, documented in the medical record, after idarucizumab infusion, during hospitalization. [c] Including all arterial and venous thromboembolic events. [d] Including adverse effects related to idarucizumab within 30 days.

We further divided the 32 patients into three subgroups. Group A contained 16 patients, including 8 with massive gastrointestinal bleedings, 4 with intracranial hemorrhages, 3 with symptomatic bleedings in a critical area, and 1 with massive hemoptysis. Group B comprised 5 patients, with 4 receiving surgeries due to intracranial hemorrhage and 1 undergoing emergent laparotomy. In Group C, patients were deemed ineligible for idarucizumab due to the reasons such as receiving intra-arterial thrombectomy (IA) or tissue plasminogen activator (TPA) infusion (45.5%, n = 5), not taking dabigatran (36.4%, n = 4), sepsis with disseminated intravascular coagulation but without active bleeding (9.1%, n = 1), and minor intramuscular bleeding (9.1%, n = 1). The study flowchart is presented in Figure 1.

*3.2. The Safety and Effectiveness Outcomes of the Patients*

Regarding the safety outcomes (Tables 1 and 2), only one thromboembolic event with ischemic stroke was reported in the eligible group and none were reported among ineligible patients, with an overall thromboembolic event rate of 3.1% (1/32). The intra-hospital mortality rate was higher in the ineligible group (27.3%) compared to the eligible group (9.5%). Of all patients who received the infusion, only one patient (3.9%) with intracranial hemorrhage experienced rebleeding after receiving idarucizumab. The overall rate of adverse events was 9.4% (n = 3), with two patients in the eligible group experiencing delirium and aspiration pneumonia, and one patient in the ineligible group experiencing pneumonia.
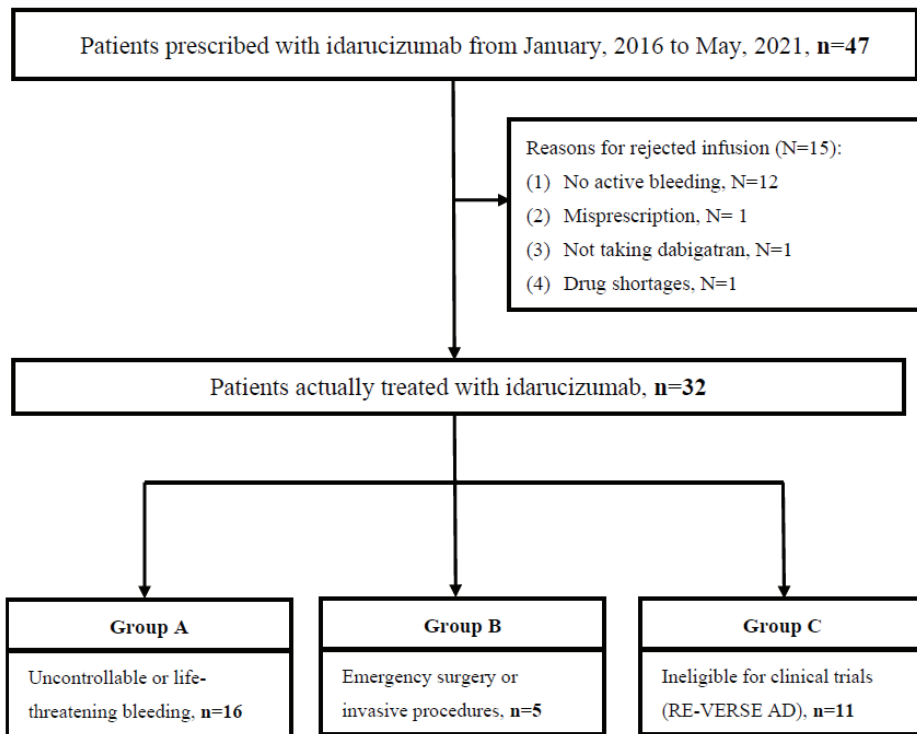
**Figure 1.** Study flowchart of the included patients.

**Table 2.** Real-world effectiveness of idarucizumab based on the major exclusion criteria of the REVERSE AD trial.

| | Patients, *n* (%) | Hemostasis, *n* (%) | Post-Infusion Bleeding, *n* (%) | Thromboembolic or Adverse Events [a], *n* (%) | Mortality [b], *n* (%) |
|---|---|---|---|---|---|
| Total patients | 32 (100%) | 24/26 (92.3%) | 1/26 (3.9%) | 1 (3.1%) | 5 (15.6%) |
| Ineligible patients | 11 (34.3%) | 4/5 (80%) | 0/5 (0%) | 0 (0%) | 3/11 (27.3%) |
| Before TPA [c] | 5 (15.6%) | N/A | N/A | 0/5 (0%) | 0/21 (0%) |
| Did not take dabigatran | 4 (12.5%) | 3/4 (75%) | 1/4 (25%) | 0/4 (0%) | 2/4 (50%) |
| Minor bleeding | 1 (3.1%) | 1/1 (100%) | 0/1 (%) | 0/1 (0%) | 0/1 (0%) |
| Sepsis without active bleeding | 1 (3.1%) | N/A | N/A | 0/1 (0%) | 1/1 (100%) |
| Eligible patients | 21 (65.6%) | 20/21 (95.2%) | 1/21 (4.8%) | 1/21 (4.8%) | 2/21 (9.5%) |

[a] Included all arterial and venous thromboembolic events. [b] Defined as intra-hospital mortality, consisting of death by any cause, documented in the medical record, after idarucizumab infusion, during hospitalization. [c] TPA: tissue plasminogen activator.

In terms of the difference in effectiveness between eligible and ineligible patients (Tables 1 and 2), the rate of successful hemostasis was higher in the eligible group (95.2% versus 80%). Furthermore, in the eligible group, 73.3% (11/15) of the patients achieved complete reversal of anticoagulant effects, with a median aPTT of 43.6 s prior to infusion of idarucizumab. However, in the ineligible group, none of the patients achieved complete reversal (0/10), with a median aPTT of 35.7 s before infusion. The detailed information is summarized in Table 3.

Table 3. Baseline characteristics and clinical outcomes in study patients.

| Patient Number | Gender (Age) | CCR, mL/min | DOAC Dosage | HASBLEED Score | CHA$_2$DS$_2$-VASc Score | Eligibility for REVERSE AD | Hemostasis | Thromboembolic or Adverse Effects | Mortality |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Group A: uncontrollable or life-threatening bleeding | | | | |
| 1 | Male (89) | 21.2 | Dabigatran, 150 mg, BID | 4 | 4 | Yes (GI bleeding) | Yes | No | Yes |
| 2 | Male (65) | 40.1 | Dabigatran, 150 mg, BID | 5 | 4 | Yes (ICH) | No | No | No |
| 3 | Female (92) | 64.1 | Dabigatran, 150 mg, BID | 2 | 8 | Yes (GIB) | Yes | No | No |
| 4 | Female (89) | 31.3 | Dabigatran, 150 mg, BID | 3 | 5 | Yes (GIB) | Yes | No | No |
| 5 | Male (68) | 56.7 | Dabigatran, 110 mg, BID | 3 | 6 | Yes (Compartment syndrome) | Yes | No | No |
| 6 | Female (84) | 50.4 | Dabigatran, 150 mg, BID | 4 | 7 | Yes (Compartment syndrome) | Yes | No | No |
| 7 | Female (85) | 72.5 | Dabigatran, 150 mg, BID | 4 | 5 | Yes (ICH) | Yes | No | No |
| 8 | Male, (87) | 58.5 | Dabigatran, 150 mg, BID | 2 | 7 | Yes (ICH) | Yes | No | No |
| 9 | Female (91) | 34 | Dabigatran, 150 mg, BID | 4 | 6 | Yes (Compartment syndrome) | Yes | No | No |
| 10 | Male (75) | 82.4 | Dabigatran, 150 mg, BID | 2 | 3 | Yes (ICH) | Yes | No | No |
| 11 | Male (76) | 83.1 | Dabigatran, 150 mg, BID | 3 | 5 | Yes (GIB) | Yes | No | No |
| 12 | Female (77) | 14.25 | Dabigatran, 110 mg, BID | 6 | 6 | Yes (Hemoptysis) | Yes | No | No |
| 13 | Female (88) | 46.8 | Dabigatran, 150 mg, BID | 3 | 7 | Yes (GIB) | Yes | No | No |
| 14 | Female (64) | 6.2 | Dabigatran, 110 mg, BID | 2 | 2 | Yes (GIB) | Yes | No | No |
| 15 | Male (82) | 39.1 | Dabigatran, 110 mg, BID | 3 | 5 | Yes (GIB) | N/A | No | Yes |
| 16 | Male (87) | 91.4 | Dabigatran, 150 mg, BID | 1 | 5 | Yes (GIB) | N/A | No | No |
| | | | | | Group B: Emergency surgery or invasive procedures | | | | |
| 17 | Female (92) | 70.4 | Dabigatran, 150 mg, BID | 1 | 3 | Yes (PPU) | Yes | Yes | No |
| 18 | Male, (70) | 56.7 | Dabigatran, 150 mg, BID | 2 | 6 | Yes (ICH) | Yes | No | No |
| 19 | Male (71) | 56.9 | Dabigatran, 150 mg, BID | 3 | 4 | Yes (ICH) | Yes | Yes | No |
| 20 | Female (71) | 105.8 | Dabigatran, 110 mg, BID | 1 | 6 | Yes (ICH) | Yes | No | No |
| 21 | Female (69) | 79 | Dabigatran, 150 mg, BID | 2 | 6 | Yes (ICH) | N/A | Yes | No |
| 17 | Female (92) | 70.4 | Dabigatran, 150 mg, BID | 1 | 3 | Yes (PPU) | Yes | Yes | No |
| | | | | | Group C: Ineligible for clinical trials (RE-VERSE AD) | | | | |
| 22 | Male (62) | 51.5 | Dabigatran, 150 mg, BID | 2 | 6 | NO (Pre-TPA/IA) | Yes | No | No |
| 23 | Female (63) | 59.6 | Dabigatran, 150 mg, BID | 3 | 7 | NO (Pre-TPA) | Yes | No | No |
| 24 | Female (70) | 87.3 | Dabigatran, 150 mg, BID | 3 | 3 | NO (Pre-TPA) | Yes | No | No |
| 25 | Male (53) | 70 | Dabigatran, 150 mg, BID | 2 | 1 | NO (Pre-TPA) | Yes | No | No |
| 26 | Male (91) | 105.9 | Dabigatran, 150 mg, BID | 3 | 4 | NO (Pre-TPA) | Yes | Yes | No |
| 27 | Male (79) | 47.9 | Rivaroxaban, 10 mg QD | 2 | 2 | NO (did not take dabigatran) | 1 | No | NO |
| 28 | Female (55) | 89.1 | Rivaroxaban, 1/4 10 mg BID | 4 | 6 | NO (did not take dabigatran) | 1 | No | Yes |
| 29 | Female (77) | 90.22 | Apixaban, 5 mg QD | 2 | 5 | NO (did not take dabigatran) | 0 | No | Yes |
| 30 | Male (64) | 34.4 | Rivaroxaban, 15 mg QD | 0 | 1 | NO (did not take dabigatran) | 1 | No | No |

**Table 3.** *Cont.*

| Patient Number | Gender (Age) | CCR, mL/min | DOAC Dosage | HASBLEED Score | CHA$_2$DS$_2$-VASc Score | Eligibility for REVERSE AD | Hemostasis | Thromboembolic or Adverse Effects | Mortality |
|---|---|---|---|---|---|---|---|---|---|
| 31 | Female (87) | 71.9 | Dabigatran, 110 mg, BID | 4 | 6 | NO (Sepsis without active bleeding) | N/A | No | Yes |
| 32 | Female (64) | 121 | Dabigatran, 150 mg, BID | 2 | 5 | NO (minor bleeding) | 1 | No | No |

## 4. Discussion

Our study revealed that 34.4% of the real-world cases of idarucizumab use would have been ineligible for inclusion in the RE-VERSE AD trials. Our findings confirmed that idarucizumab is safe and effective for Taiwanese patients who meet the eligibility criteria of the RE-VERSE AD trials, as well as for acute ischemic stroke patients facing emergent interventions who would have been ineligible. On the other hand, our results also suggested that the safety profile of idarucizumab is comparable, but its effectiveness may be limited, in other patient groups who would have been ineligible for the trials.

In this retrospective study, we aimed to evaluate the effectiveness and safety outcomes of idarucizumab in an Asian population, stratified by eligibility for participation in the RE-VERSE AD trial. The eligible and ineligible groups had similar sex distribution, body weight, and daily dose of dabigatran, but the eligible group was older (79.6 vs. 69.5 years old) and had a higher previous systemic embolism rate (23.8% vs. 9.1%) and a higher baseline median aPTT value (43.2 vs. 35.7 s). The overall successful hemostasis rate among the potentially eligible patients was high (95.2%), with a considerable rate of complete reversal of anticoagulant effects (73.3%) and excellent safety outcomes, including a low thromboembolic event rate (4.8%), rebleeding rate (4.8%), intra-hospital mortality rate (9.5%) and adverse event rate (9.5%), compared to the original RE-VERSE AD trials. Thus, consistent with previous studies, idarucizumab generally provided instant and effective reversal of anticoagulant effects with few post-infusion side effects, regardless of race [11,13,15–17,32,33]. By contrast, among ineligible patients, the successful hemostasis rate (80%) and reversal of anticoagulant effects (0%) were less prominent, despite a low adverse event rate (9.1%), rebleeding rate (0%), and thromboembolic event rate (0%). Nonetheless, among these ineligible patients, dabigatran-treated acute ischemic stroke patients were able to safely receive intravenous thrombolysis or intravenous tissue plasminogen activator treatment after the infusion with idarucizumab.

For dabigatran-treated acute ischemic stroke patients, hemorrhagic transformation was the major concern before definite intervention such as intravenous thrombolysis or intravenous tissue plasminogen activator treatment. In our study, around half of the trial-ineligible patients were acute ischemic stroke patients who qualified for intravenous thrombolysis (n = 1, 20%) or intravenous tissue plasminogen activator treatment (n = 5, 100%). The median initial NIHSS score was 13.2, and all had uneventful courses except for one patient who developed subsequent aspiration pneumonia. The average time from the infusion to the definite intervention was 29 min. A recent systematic review of dabigatran-treated patients infused with idarucizumab before intravenous thrombolysis or intravenous tissue plasminogen activator demonstrated the effectiveness and safety of this therapeutic strategy. The review found favorable outcomes regarding the rate of hemorrhagic transformation and mortality compared to non-anticoagulated patients [21,23,26,34]. Our findings are comparable to the results of previous studies in which idarucizumab was not only a feasible therapeutic strategy but also saved valuable time for the subsequent definite treatments.

Our study also included other trial-ineligible patients from previous trials. A total of 11 patients were found to be ineligible for the study. Among these 11, 4 patients were not eligible due to not taking dabigatran (three were taking rivaroxaban, and one was taking

apixaban), whereas 2 patients were not experiencing life-threating bleeding. In addition, 5 patients taking dabigatran received tissue plasminogen activator (TPA) for acute ischemic stroke. Despite the low case numbers, the findings in these patients are consistent with the fact that idarucizumab is the specific reversal agent for dabigatran—higher mortality rate (60%), lower successful hemostasis rate (80%), and low complete anticoagulant reversal rate (0%). Therefore, the assumption that this therapeutic strategy may have limited value in this non-qualified population is reasonable [14]. Further research is required to determine the efficacy of idarucizumab in this subset of patients, given the small sample size of our study.

Among the 47 patients who were prescribed idarucizumab, 12 (25.5%) were atrial fibrillation patients who underwent radiofrequency catheter ablation. The infusion of idarucizumab was not performed because there was no uncontrolled major bleeding during the intervention. Given the high risk of bleeding during the procedure, which involves transseptal puncture and ablation of the left atrium for pulmonary vein isolation, the anticoagulant strategy for patients with atrial fibrillation who undergo catheter ablation merits attention. Uninterrupted dabigatran is one of the preferred anticoagulant strategies, not only because it has fewer bleeding complications, but also because specific reversal agents are available [35–39]. The hemostasis rate of idarucizumab in an uncontrolled bleeding situation during catheter ablation is around 80%, with few adverse effects reported [38,40]. Thus, despite none of the patients who underwent radiofrequency catheter ablation actually being administered idarucizumab, uninterrupted dabigatran, with prepared idarucizumab on standby for emergency situations appears feasible in clinical settings which were not studied in the RE-VERSE AD trials.

In terms of adverse effects in real-world settings, the most common relevant side effects include delirium (7%), constipation (7%), pyrexia (6%) and pneumonia (6%) [14]. In comparison to the RE-VERSE AD trials where 23.3% (117/503) of the enrolled patients reported side effects, our study had a lower incidence, at 9.4% (3/32), with only one patient experiencing delirium and two patients developing aspiration pneumonia. Thromboembolic events were also a major concern due to the rebound effect. In the RE-VERSE AD trials, the thromboembolic event rate was 4.8% (24/503), whereas in our study, only a single event was found. This patient had atrial fibrillation and left middle cerebral artery infarction with hemorrhage transformation, in 2017, while taking dabigatran 150 mg twice daily to prevent ischemic stroke. In May 2021, the patient was prescribed idarucizumab due to left chronic subdural hemorrhage with midline shift (3.5 mm). Following treatment, the patient was discharged and did not use dabigatran or other anticoagulants after discharge. However, the patient experienced left-sided weakness and a recurrent ischemic stroke in the right temporo-occipital area and right subacute SDH with midline shift (10.2 mm), as revealed by a magnetic resonance imaging conducted on 23 June 2021. The patient received subdural drainage and recovered well without focal neurological signs. Eventually, the patient received dabigatran 110 mg twice daily again and refused left atrial appendage occlusion after discussion with a cardiovascular doctor. The time interval between the infusion of idarucizumab and the onset of right temporo-occipital ischemic stroke was 35 days. In our study, we found a lower rate of 90-day thromboembolic events, 3.1%. Additionally, a recent meta-analysis reported a pooled thromboembolic event rate of around 5.5% over 90 days in patients treated with a specific antidote [15,33]. Therefore, despite the proven safety record of idarucizumab, close monitoring for possible adverse effects is necessary in real-world settings.

Patients with atrial fibrillation commonly have coronary artery disease, and treating them with anticoagulants combined with antiplatelet therapy can be complex and challenging. Dual antiplatelet therapy (DAPT) is necessary for acute coronary syndrome, or stenting for coronary artery disease, and oral anticoagulants for stroke prevention are indicated in these patients due to a $CHA_2DS_2$-VASc score of at least 1, as well as the simultaneous presence of other cardiovascular risk factors. The 2021 European Heart Rhythm Association Practical Guide suggests that the use of direct oral anticoagulant (DOAC) combined

with DAPT for up to 30 days may be advisable in patients with a high atherothrombotic risk, followed by a shift to therapy with DOAC combined with P2Y12 inhibitor for six months to one year [39]. However, the guideline also emphasizes the need to individualize the duration of combined therapy of DAPT and DOAC based on atherothrombotic and bleeding risk, as the risk of bleeding is expectedly elevated when anticoagulants are combined with antiplatelet therapy, creating a dilemma in clinical practice when the patient experiences life-threatening bleeding or requires emergency surgery. Additionally, the specific combination of drugs used in triple therapy may have an impact on the risk of bleeding complications [41]. To our knowledge, only two case reports have discussed the use of idarucizumab in this condition, and there are no trials addressing this issue [42,43]. Despite few case reports discussing the use of idarucizumab in patients with both atrial fibrillation and coronary artery disease, it may be a useful option in these patients who face life-threatening bleeding or require emergency surgery. Further research is needed to better understand how the composition of triple therapy affects the incidence and severity of bleeding complications and to evaluate the efficacy and safety of idarucizumab in this specific population.

The major strength of our study is that it provides comprehensive results for an idarucizumab-treated population and compares the effectiveness and safety outcomes with respect to patients' trial eligibility, which has seldom been reported before. Relatively few studies have investigated the effectiveness and safety of idarucizumab in an Asian population. However, some limitations remain to our study. First, our study is retrospective, which means it could be subject to selection bias and confounding factors. Second, due to the relative infrequency of idarucizumab infusion, and despite utilizing the largest multi-institutional database in Taiwan, we were only able to enroll a smaller number of patients compared to previous trials. This limitation may restrict the generalizability of our findings and there may be differences in patient characteristics or treatment practices across different institutions. Third, the unknown duration between the last administration of dabigatran and the infusion of idarucizumab may have influenced our assessment of the effectiveness of idarucizumab. However, this aspect brings our study closer to real-world clinical conditions. Fourth, since our study lacked a control arm, it is not possible to make a direct comparison. As a result, the findings should be interpreted with caution. Fifth, in our study, the infusion rate of idarucizumab was not recorded in our medical records. Consequently, we were unable to gather additional information regarding the infusion rate and make comparisons to previous studies regarding safety and efficacy outcomes. Sixth, the timing of aPTT measurement varied in our study, with most of the data collected covering hours to days, or some data not being collected at all. In addition, some exact times of idarucizumab infusion were not available. The variability in the timing of aPTT measurements and the lack of exact timings of idarucizumab infusion made it difficult for us to assess the onset of the drug's reversal effect, resulting in some inaccuracy in the determination of successful hemostasis rates. Finally, due to the nature of the study, several important issues were not addressed, such as choice of anticoagulants for resumption after the infusion.

## 5. Conclusions

In Taiwan, 34.4% of real-world cases of idarucizumab use would have been ineligible for participation in the initial safety and efficacy trials. However, our study has demonstrated the real-world effectiveness and safety of administration of idarucizumab among those who would have been eligible for the trials, as well as among acute ischemic stroke patients, regardless of their eligibility for the trials. In contrast, for trial-ineligible patients, although idarucizumab administration seems to be safe, it appears to be less effective. Our study provided further evidence for extending the applicability of idarucizumab in real-world scenarios.

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*Article*

# The Role Played by Novel Inflammatory Markers in Assessment of Peripheral Artery Disease

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**Abstract:** *Background and Objectives*: Atherosclerosis is a multifactorial process in which inflammatory markers have both therapeutic and prognostic roles. Recent studies bring into question the importance of assessing new inflammatory markers in relation to the severity of peripheral artery disease (PAD), such as the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and lymphocyte-to-C-reactive protein ratio (LCR). *Materials and Methods*: We conducted a retrospective and descriptive study including 652 patients with PAD, who were divided into two groups according to the severity of the ankle–brachial index value: mild and moderate obstruction (257 patients) and severe obstruction (395 patients). We evaluated demographics, anthropometric data and clinical and paraclinical parameters in relation to the novel inflammatory biomarkers mentioned above. *Results*: Weight ($p = 0.048$), smoking ($p = 0.033$), the number of cardiovascular risk factors ($p = 0.041$), NLR ($p = 0.037$), LCR ($p = 0.041$) and PLR ($p = 0.019$), the presence of gangrene ($p = 0.001$) and the number of lesions detected via peripheral angiography ($p < 0.001$) were statistically significant parameters in our study. For the group of patients with severe obstruction, all three inflammatory biomarkers were statistically significantly correlated with a serum low-density lipoprotein–cholesterol level, the number of cardiovascular risk factors, rest pain, gangrene and a risk of amputation. In addition, directly proportional relationships were found between NLR, PLR and the number of stenotic lesions ($p = 0.018$, $p = 0.016$). Also, NLR (area under the curve <AUC> = 0.682, $p = 0.010$) and PLR (AUC = 0.692, $p = 0.006$) were predictors associated with a high risk of amputation in patients with an ABI < 0.5. *Conclusions*: in our study, we demonstrated the importance of assessing inflammatory markers in relation to the presence of cardiovascular risk factors through the therapeutic and prognostic value demonstrated in PAD.

**Keywords:** peripheral artery disease; biomarkers; inflammation; cardiovascular risk; neutrophil-to-lymphocyte ratio; platelet-to-lymphocyte ratio; lymphocyte-to-C-reactive protein ratio

## 1. Introduction

Peripheral arterial disease (PAD) is one of the main atherosclerotic cardiovascular diseases, and its prevalence has increased, despite the large-scale implementation of primary prevention strategies in recent years [1]. More than 50% of patients with PAD are asymptomatic, leading to a high rate of complications related to increased morbidity and mortality in the absence of multidisciplinary and integrative management approaches used to reduce the risk of a potentially fatal acute vascular event [2–4].

In general, 3–4% of amputations that occur annually have obstructive atherosclerotic lesions as their morphopathological substrate, leading to negative prognostic effects in the medium and long term. From a pathophysiological point of view, the atherosclerotic process has a multifactorial origin, with inflammation playing an important role in mediating the processes involved in the progression and destabilization of atherosclerosis [5,6].

PAD is frequently associated with both classic and new cardiovascular risk factors. Of the latter factor type, the pro-inflammatory status, through new inflammatory markers with anti-inflammatory roles, has attracted the interest of the scientific community both in terms of its potential prognostic role and future therapeutic targets among patients with PAD and HF.

The complete blood count is one of the most common biological determinations, and it can provide details associated with the presence of a pro-inflammatory status [2,7]. Recent studies published in the literature have demonstrated the prognostic roles of several inflammatory markers derived from complete blood count and lipid profile analysis performed in these patients. The neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), white blood cells-to-mean platelet volume ratio (MPV), and lymphocyte-to-C-reactive protein ratio (LCR) are potential inflammatory biomarkers with prognostic value among patients with PAD [8]. The systemic immune–inflammation index [9], monocyte-to-high-density lipoprotein (HDL) cholesterol ratio and lymphocyte-to-HDL ratio [10] are biomarkers with important roles in atherogenesis, in addition to those previously mentioned roles.

The possibility that easy and accessible dosing of these markers can provide meaningful clues regarding the patient's evolution is the main incentive to explore them. The studies available in the literature to date have separately addressed the issue of pro-inflammatory status in relation to PAD or cardiovascular risk in general.

Recent data from the literature bring to light a number of new, easy-to-use and reproducible inflammatory molecules, such as the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and lymphocyte-to-C-reactive protein ratio (LCR) [11,12]. Their use as markers associated with the presence of cardiovascular risk factors or severity of obstruction and clinical picture of patients with PAD is limited to date, which justifies the current study. NLR and PLR have previously been shown to be predictors of the risk of an acute vascular event occurring [10,13]. These molecules may also serve as future therapeutic targets for these patients, as the prognostic role of anti-inflammatory medication in patients with atherosclerotic disease has previously been demonstrated in numerous clinical trials [14,15]. Nucleotide oligomerization domain-like receptor family, pyrin domain containing 3 (NLRP3) and interleukin 6 (IL-6) are other inflammatory markers that have a demonstrated role in modulating the inflammatory processes involved in the development and progression of atherosclerosis [16]. In the case of NLRP3, previous clinical research has demonstrated the existence of elevated serum levels of this marker in patients with PAD, together with evidence of a correlation between this marker, macrophage accumulation and the degree of calcification of the arteries [17].

In this study, we aim to identify a series of clinico-biological particularities by analyzing a group of patients diagnosed with PAD, as well as assess the efficiency and efficacy of using new inflammatory biomarkers, and, therefore, provide practicing cardiologists with a feasible and easy-to-apply tool with both prognostic and therapeutic roles.

## 2. Materials and Methods

### 2.1. Study Design

We conducted a retrospective and descriptive study involving 688 consecutive patients diagnosed with PAD and evaluated in the Cardiology Department of "St. Spiridon" Hospital. Thirty-six patients were excluded from the initial group due to incomplete medical records (angiographic evaluation or biological parameters). Thus, the final study group consisted of 652 patients with PAD evaluated in a multidisciplinary manner (Figure 1).
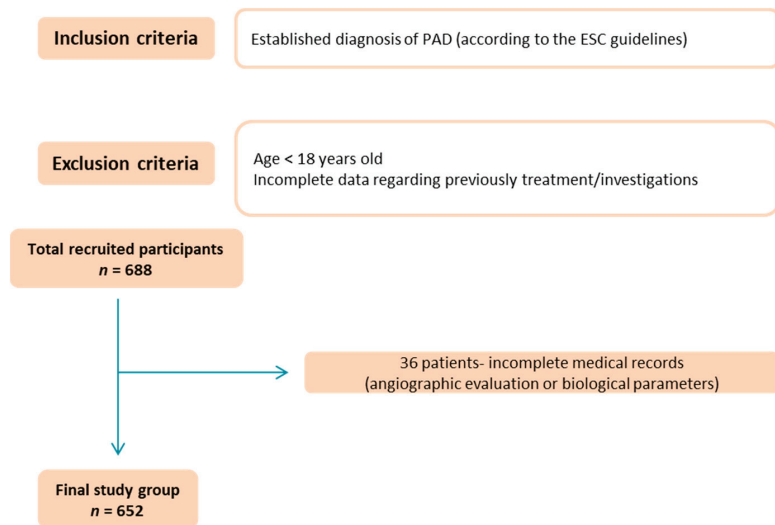


**Figure 1.** Flow chart of the studied group (PAD: peripheral artery disease; ESC: European Society of Cardiology).

Inclusion criteria were being over 18 years of age, a definite diagnosis of PAD according to the clinical guidelines of the European Society of Cardiology [18] and the provision by those who wished to participate in this study of signed and informed consent. Exclusion criteria were being under 18 years of age, a lack of informed consent and incomplete medical records.

In the absence of a definite diagnosis of PAD established via vascular Doppler ultrasound or peripheral angiography, the presence of symptoms suggestive of PAD was assessed. Symptoms suggestive for PAD were intermittent claudication (IC), presence of paresthesia in the lower limbs, a lack of pilosity, cold and pale skin, petechiae and the presence of dermatitis or ulcers caused by decreased vascularity.

### 2.2. Measurements

#### 2.2.1. Comorbidities and Laboratory Data

We included demographic, anthropometric and paraclinical (biological and imaging) parameters in our study. Anamnesis revealed the presence of major cardiovascular risk factors, such as hypertension [19], diabetes mellitus [20], dyslipidemia [21], smoking, obesity and a sedentary lifestyle. Smoking was quantified as "pack years", with a pack year being measured as 20 cigarettes being smoked daily for one year [22].

Medical data regarding demographics, personal medical history, tobacco, alcohol consumption habits and chronic medication were obtained from the observation charts. Body mass index (BMI) was calculated as the ratio of weight (kg) to height (m²).

A calibrated medical scale was used to assess the body weights of patients included in the study according to international standards. The measurement was performed for each patient on an unweighted basis, with patients removing clothing considered likely

to generate significant weight fluctuations. The blood pressure profile was assessed in all patients enrolled in the study, with the main components used in the statistical analysis being systolic blood pressure (SBP, mmHg), diastolic blood pressure (DBP, mmHg) and pulse pressure (PP, mmHg).

The parameters of lipid (total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides) and carbohydrate profile (serum glucose), inflammatory markers (serum fibrinogen) and renal function (serum creatinine and urea) were evaluated. In addition to the biological parameters usually evaluated in all patients with associated cardiovascular pathologies, based on complete blood counts, we calculated a series of new biomarkers with proven roles, as shown in the literature, in the assessment of inflammatory status, such as NLR, PLR and LCR. NLR was calculated as the ratio of absolute neutrophil (N) to lymphocyte (L) values. PLR was calculated as the ratio of absolute platelets (P) to L values. LCR ratio was calculated as the ratio of absolute L to hs-CRP values. All results were presented according to the International System of Units.

### 2.2.2. Transthoracic Echocardiography

Transthoracic echocardiography was performed at the first evaluation based on European guidelines (European Association of Cardiovascular Imaging) related to the purpose of the functional and morphological assessment of the heart [23]. All imaging examinations were performed using the same echocardiograph (Toshiba Aplio 500 Series, Toshiba Medical Systems Corporation, Ōtawara, Tochigi, Japan) by the same experienced cardiologist. Left ventricle (LV) systolic function was assessed by calculating the LV ejection fraction (LVEF) via the Simpson biplane method.

### 2.2.3. Angiography

Peripheral angiography is the gold standard method of diagnosis and treatment in PAD. Prior to the procedure, all patients were informed of the risks and potential complications associated with the minimally invasive procedure. Biological samples were taken from all patients (especially renal function, complete blood count, blood group, and hemostasis parameters), and a venous line was fitted. In diabetic patients receiving Metformin treatment, it was recommended to discontinue treatment 24 h prior to the procedure and resume it after 48 h to reduce the risk of associated nephrotoxicity.

The angiography technique is the standard method used in all interventional cardiology centers [24,25]. Contrast medium injection was performed, allowing the visualization of the arterial system at the aorto-iliac, femuro-popliteal and infra-popliteal levels. In patients with stenotic lesions with indication of interventional revascularization, this procedure was performed in accordance with clinical protocols. All angiograms were performed by the same cardiologist. The severity of atherosclerotic lesions and their indication for interventional revascularization were determined using The Global Limb Anatomic Staging System (GLASS) [26]. The risk of amputation was assessed using the WIfl classification, taking into account the trophic lesions present, as well as ischemia or associated leg infections. The WIfl classification takes into account the presence of the three main components of trophic lesions, signs of ischemia and the presence of infection in the foot (scored from 0 to 3 points depending on their severity). As for the trophic lesion, it was quantified as follows: 0—rest pain, no ulcer; 1—small, superficial ulcer, located distal or at the level of the foot, without gangrene; 2—deep ulcer with exposure of bone, joint or tendon, possibly with gangrene limited to the toes; 3—deep, extensive ulcer affecting the calf, possibly with calcaneal or extensive gangrene. In case of ischemia, 0 points indicated an ABI greater than or equal to 0.8, an ankle BP greater than 100 mmHg and a halo BP greater than 60 mmHg; 1 indicated an ABI between 0.6 and 0.79, an ankle BP between 70 and 100 mmHg and a halo BP between 40 and 59 mmHg; 2 indicated an ABI between 0.4 and 0.59, an ankle BP between 50 and 70 mmHg and a halo BP between 30 and 39 mmHg; and 3 indicated an ABI below 0.4, an ankle BP below 50 mmHg and a halo BP below 30 mmHg. Foot infection was quantified as follows: 0—no signs or symptoms of infection; 1—local cutaneous and

subcutaneous cellular tissue infection; 2—deeper local infection than the previous category; 3—systemic inflammation present [27].

### 2.3. Statistical Analysis

We used the Statistical Package for the Social Science (SPSS) statistics software (version 26 for Windows; SPSS Inc., Chicago, IL, USA) to perform statistical analysis of the parameters presented above. The results obtained were reported as mean ± standard deviation (SD) for the numerical parameters or frequency and percentages for categorical parameters. We tested the normal distribution of the data using the Kolmogorov–Smirnov test. The independent T-test and ANOVA (one way analysis of variance) were used to perform the analysis of continuous variables. Pearson's and Spearman's (r) correlation coefficients were used to test the reliability of statistically significant correlations identified in our study. A *p*-value of ≤0.05 was considered to be the threshold of statistical significance. The results are presented in Tables 1 and 2. Receiver operating characteristic (ROC) analysis was performed to calculate the area under the curve for the biomarkers included in the study in order to identify predictors associated with severe obstructions. The Bonferroni Correction Method was used to perform multiple testing. Twelve tests were performed for each subgroup of patients, ensuring that for the data presented in Table 3, the *p*-value considered to be statistically significant was 0.0041.

**Table 1.** Demographics, anthropometric parameters, biological data and exercise stress test parameters.

| Parameter | Total Group (*n* = 652) | Mild and Moderate Obstruction (*n* = 257) | Severe Obstruction (*n* = 395) | *p* |
|---|---|---|---|---|
| **Demographics** | | | | |
| Age | 66.46 ± 10.47 | 65.39 ± 11.06 | 67.18 ± 10.32 | 0.333 |
| Males | 552 (84.7%) | 217 (84.44%) | 335 (84.81%) | 0.897 |
| Area of residence—urban | 273 (41.9%) | 106 (41.25%) | 167 (42.28%) | 0.794 |
| **Anthropometric data** | | | | |
| Height. M | 1.92 ± 6.4 | 1.67 ± 0.05 | 2.06 ± 7.98 | 0.435 |
| Weight. Kg | 75.94 ± 9.15 | 69.78 ± 9.66 | 81.20 ± 10.89 | 0.048 |
| BMI. Kg/m$^2$ | 26.21 ± 3.01 | 25.10 ± 2.99 | 27.15 ± 3.16 | 0.053 |
| **Vitals** | | | | |
| HR. bpm | 74.12 ± 13.96 | 73.87 ± 14.55 | 75.11 ± 16.38 | 0.076 |
| Systolic BP. mmHg | 141.93 ± 14.89 | 140.78 ± 15.05 | 142.49 ± 14.37 | 0.069 |
| Diastolic BP. mmHg | 80.15 ± 7.66 | 79.54 ± 8.34 | 80.58 ± 7.03 | 0.068 |
| Mean BP. mmHg | 100.74 ± 8.93 | 99.96 ± 9.36 | 101.22 ± 8.40 | 0.040 |
| Pulse pressure. mmHg | 73.56 ± 12.99 | 71.54 ± 11.73 | 75.81 ± 14.93 | <0.001 |
| **Cardiovascular risk factors & comorbidities** | | | | |
| Smoking | 435 (66.72%) | 184 (71.60%) | 251 (63.54%) | 0.033 |
| Smoking—packs smoked per year | 23.69 ± 18.43 | 25.63 ± 19.71 | 22.99 ± 18.94 | 0.043 |
| Dyslipidemia | 350 (53.68%) | 149 (57.98%) | 201 (50.89%) | 0.076 |
| Diabetes mellitus | 213 (32.67%) | 93 (36.19%) | 120 (30.38%) | 0.226 |
| Hypercholesterolemia (>200 mg/dL) | 267 (40.95%) | 113 (43.97%) | 154 (38.99%) | 0.206 |
| Hypercholesterolemia (>250 mg/dL) | 67 (10.28%) | 28 (10.89%) | 39 (9.87%) | 0.675 |
| HDL cholesterol < 40 mg/dL | 244 (37.42%) | 98 (38.13%) | 80 (20.25%) | 0.949 |
| LDL cholesterol > 130 mg/dL | 276 (42.33%) | 111 (43.19%) | 165 (41.77%) | 0.720 |
| Hypertriglyceridemia | 35 (5.37%) | 15 (5.84%) | 20 (5.06%) | 0.857 |
| Overweight | 51 (70.8%) | 21 (67.7%) | 30 (73.2%) | |
| Obesity class I | 18 (25.0%) | 9 (29.0%) | 9 (22.0%) | 0.762 |
| Obesity class II | 3 (4.2%) | 1 (3.2%) | 2 (4.9%) | |

**Table 1.** *Cont.*

| Parameter | Total Group (*n* = 652) | Mild and Moderate Obstruction (*n* = 257) | Severe Obstruction (*n* = 395) | *p* |
|---|---|---|---|---|
| Hypertension | 315 (48.31%) | 130 (50.58%) | 185 (46.84%) | 0.479 |
| Number of risk factors | | | | |
| 0 | 16 (2.5%) | 7 (2.7%) | 9 (2.3%) | |
| 1 | 180 (27.6%) | 66 (25.7%) | 114 (28.9%) | |
| 2 | 238 (36.6%) | 84 (32.7%) | 154 (39.1%) | |
| 3 | 156 (24.0%) | 70 (27.2%) | 86 (21.8%) | 0.041 |
| 4 | 45 (6.9%) | 23 (8.9%) | 22 (5.6%) | |
| 5 | 16 (2.5%) | 7 (2.7%) | 9 (2.3%) | |
| Cerebrovascular disease | 51 (7.82%) | 23 (8.95%) | 28 (7.09%) | 0.387 |
| **Biological data** | | | | |
| Total cholesterol. mg/dL | 198.47 ± 46.57 | 196.92 ± 46.65 | 194.36 ± 45.85 | 0.606 |
| LDL cholesterol. mg/dL | 126.82 ± 40.30 | 127.19 ± 40.70 | 126.50 ± 39.91 | 0.607 |
| HDL cholesterol. mg/dL | 41.65 ± 10.60 | 41.79 ± 11.33 | 41.25 ± 9.94 | 0.949 |
| Triglycerides. mg/dL | 135.45 ± 71.79 | 139.67 ± 80.04 | 133.06 ± 65.98 | 0.791 |
| Serum creatinine. mg/dL | 0.96 ± 0.36 | 1.07 ± 0.38 | 1.05 ± 0.36 | 0.973 |
| Serum urea. mg/dL | 44.71 ± 18.92 | 46.31 ± 21.70 | 43.74 ± 17.60 | 0.297 |
| Creatinine clearance. mL/min/1.73 m$^2$ | 62.49 ± 22.01 | 62.84 ± 22.68 | 62.16 ± 21.47 | 0.685 |
| Fasting glucose. mg/dL | 118.49 ± 48.90 | 121.93 ± 49.97 | 117.32 ± 48.75 | 0.186 |
| Serum fibrinogen. mg/dL | 395.59 ± 132.22 | 369.47 ± 115.96 | 414.71 ± 137.97 | 0.001 |
| hs-CRP. mg/dL | 6.34 ± 2.78 | 4.97 ± 3.01 | 7.07 ± 3.83 | 0.023 |
| Hematocrit. % | 41.74 ± 5.16 | 41.43 ± 5.47 | 41.85 ± 5.17 | 0.125 |
| Platelets (×10$^3$/mL) | 297.44 ± 11.17 | 281.73 ± 94.37 | 308.63 ± 119.14 | 0.018 |
| NLR | 3.21 ± 2.16 | 2.17 ± 1.75 | 4.20 ± 0.89 | 0.037 |
| LCR | 8.75 ± 13.22 | 6.21 ± 12.34 | 7.15 ± 16.37 | 0.041 |
| PLR | 138,280 ± 71,678.82 | 133,560.11 ± 60,047.82 | 145,181.91 ± 70,236.67 | 0.019 |
| **Clinical parameters** | | | | |
| Pain at rest | 541 (81.44%) | 191 (74.32%) | 350 (88.61%) | <0.001 |
| Erythema | 77 (11.81%) | 30 (11.67%) | 47 (11.90%) | 0.930 |
| Ulcerations | 93 (14.26%) | 34 (13.23%) | 59 (14.94%) | 0.542 |
| Necrosis | 27 (4.14%) | 6 (2.33%) | 21 (5.32%) | 0.062 |
| Gangrene | 121 (18.51%) | 29 (11.28%) | 92 (23.29%) | 0.001 |
| Bilateral clinical involvement | 231 (35.43%) | 82 (31.91%) | 149 (37.72%) | 0.052 |
| Cardiac murmurs | 119 (18.25%) | 51 (19.84%) | 68 (17.22%) | 0.271 |
| Femoral artery murmur | 149 (22.85%) | 50 (19.46%) | 99 (25.06%) | 0.175 |
| Carotid artery murmur | 77 (11.81%) | 34 (13.23%) | 43 (10.89%) | 0.484 |
| Renal artery murmur | 24 (3.68%) | 9 (3.50%) | 15 (3.80%) | 0.707 |
| **Rutherford classification** | | | | |
| Class 3 | 106 (16.3%) | 66 (25.7%) | 40 (10.2%) | |
| Class 4 | 213 (32.7%) | 89 (34.6%) | 123 (31.2%) | <0.001 * |
| Class 5 | 205 (31.4%) | 65 (25.3%) | 140 (35.5%) | |
| Class 6 | 128 (19.6%) | 37 (14.4%) | 91 (23.1%) | |
| **Leriche–Fontaine classification** | | | | |
| 2nd stage | 105 (16.1%) | 64 (24.9%) | 41 (10.13%) | |
| 3rd stage | 212 (32.5%) | 121 (47.08%) | 91 (23.03%) | <0.001 * |
| 4th stage | 335 (51.4%) | 72 (28.01%) | 263 (66.58%) | |
| **Paraclinical data** | | | | |
| Arterial Doppler US | 110 (16.95%) | 28 (10.89%) | 81 (20.51%) | 0.004 |
| Angio MRI | 32 (4.9%) | 10 (3.89%) | 22 (5.57%) | 0.448 |
| Arteriography | 635 (97.4%) | 252 (98.05%) | 382 (96.71%) | 0.305 |

**Table 1.** *Cont.*

| Parameter | Total Group (*n* = 652) | Mild and Moderate Obstruction (*n* = 257) | Severe Obstruction (*n* = 395) | *p* |
|---|---|---|---|---|
| Number of lesions (stenosis and thrombosis) | | | | |
| 0 | 5 (0.8%) | 4 (1.56%) | 4 (0.25%) | |
| 1 | 226 (34.7%) | 97 (37.74%) | 129 (32.65%) | |
| 2 | 183 (28.1%) | 78 (30.35%) | 105 (26.58%) | <0.001 |
| 3 | 98 (15.0%) | 40 (15.56%) | 58 (14.68%) | |
| 4 | 65 (10.0%) | 15 (5.84%) | 50 (12.66%) | |
| 5 | 40 (6.1%) | 11 (4.28%) | 29 (7.34%) | |
| ≥6 | 35 (4.6%) | 10 (4.27%) | 23 (5.83%) | |
| LVEF. % | 57.36 ± 10.08 | 58.20 ± 9.95 | 56.66 ± 10.17 | 0.057 |
| **Therapeutic management** | | | | |
| Medical | 650 (99.8%) | 257 (100.0%) | 393 (99.49%) | 0.521 |
| Interventional revascularization | 48 (7.36%) | 31 (12.06%) | 17 (4.30%) | <0.001 |
| Surgical revascularization | 369 (56.6%) | 132 (51.36%) | 236 (59.75%) | 0.061 |
| Risk of amputation | 210 (32.1%) | 62 (24.12%) | 148 (37.47%) | <0.001 |

All values are expressed as mean ± standard deviation (SD). LDL cholesterol: low-density lipoprotein cholesterol, HDL cholesterol: high-density lipoprotein cholesterol; hs-CRP: high-sensitivity C-reactive protein (normal range 0–1 mg/dL); HbA1C: glycated hemoglobin; NLR: neutrophil-to-lymphocyte ratio, normal range 0.43–2.75 in males and 0.37–2.87 in females; LCR: lymphocyte-to-C-reactive protein ratio, normal range—not defined; PLR: platelet-to-lymphocyte ratio, normal range 36.63–149.13 in males and 43.36–172.68 in females; HR: heart rate; BP: blood pressure; ABI: ankle–brachial index; US: ultrasonography; MRI: magnetic resonance imaging; LVEF: left ventricle ejection fraction. * The *p*-value was assessed on the basis of walking distances until the onset of intermittent claudication.

**Table 2.** Biological parameters based on the number of associated cardiovascular risk factors.

| Parameter | Mild and Moderate Obstruction (*n* = 257) | Severe Obstruction (*n* = 395) | *p* |
|---|---|---|---|
| **No Cardiovascular Risk Factors** | | | |
| Total cholesterol, mg/dL | 202.11 ± 28.03 | 199.86 ± 46.12 | 0.882 |
| LDL cholesterol, mg/dL | 136.31 ± 22.56 | 125.89 ± 35.95 | 0.806 |
| HDL cholesterol, mg/dL | 41.78 ± 3.99 | 40.29 ± 11.28 | 0.612 |
| Triglycerides, mg/dL | 120.11 ± 42.08 | 168.43 ± 66.50 | 0.144 |
| Serum creatinine, mg/dL | 0.92 ± 0.15 | 1.20 ± 0.71 | 0.420 |
| Serum urea, mg/dL | 38.56 ± 13.68 | 55.86 ± 45.27 | 0.057 |
| Fasting glucose, mg/dL | 90.44 ± 7.54 | 95.43 ± 8.72 | 0.436 |
| Serum fibrinogen, mg/dL | 406.11 ± 107.53 | 478.57 ± 136.37 | 0.624 |
| NLR | 2.22 ± 1.89 | 2.46 ± 2.04 | 0.169 |
| LCR | 6.05 ± 11.63 | 7.17 ± 11.78 | 0.247 |
| PLR | 134,115 ± 59,753.70 | 138,098 ± 71,367.71 | 0.079 |
| **1 cardiovascular risk factor** | | | |
| Total cholesterol, mg/dL | 203.61 ± 55.30 | 198.61 ± 46.03 | 0.576 |
| LDL cholesterol, mg/dL | 133.90 ± 48.42 | 129.50 ± 40.98 | 0.690 |
| HDL cholesterol, mg/dL | 42.03 ± 10.85 | 41.70 ± 10.11 | 0.497 |
| Triglycerides, mg/dL | 138.39 ± 62.06 | 137.03 ± 65.79 | 0.772 |
| Serum creatinine, mg/dL | 1.01 ± 0.42 | 1.06 ± 0.42 | 0.399 |
| Serum urea, mg/dL | 41.95 ± 17.85 | 43.20 ± 20.82 | 0.785 |
| Fasting glucose, mg/dL | 96.25 ± 24.22 | 98.65 ± 20.82 | 0.113 |
| Serum fibrinogen, mg/dL | 396.48 ± 145.22 | 361.08 ± 118.96 | 0.135 |
| NLR | 2.31 ± 1.67 | 2.43 ± 2.89 | 0.083 |
| LCR | 5.98 ± 10.85 | 6.48 ± 9.45 | 0.062 |
| PLR | 129,774.51 ± 53,782.3 | 134,781 ± 51,741 | 0.108 |

**Table 2.** *Cont.*

| Parameter | Mild and Moderate Obstruction (*n* = 257) | Severe Obstruction (*n* = 395) | *p* |
|---|---|---|---|
| **2 cardiovascular risk factors** | | | |
| Total cholesterol, mg/dL | 191.23 ± 44.57 | 203.15 ± 49.21 | 0.457 |
| LDL cholesterol, mg/dL | 124.63 ± 36.81 | 130.56 ± 43.13 | 0.848 |
| HDL cholesterol, mg/dL | 40.74 ± 9.02 | 41.86 ± 14.25 | 0.749 |
| Triglycerides, mg/dL | 129.31 ± 66.12 | 153.67 ± 111.67 | 0.168 |
| Serum creatinine, mg/dL | 1.05 ± 0.34 | 1.05 ± 0.35 | 0.754 |
| Serum urea, mg/dL | 43.94 ± 18.28 | 45.12 ± 18.11 | 0.801 |
| Fasting glucose, mg/dL | 115.73 ± 50.02 | 112.27 ± 41.37 | 0.064 |
| Serum fibrinogen, mg/dL | 430.95 ± 137.66 | 349.19± 99.69 | <0.001 |
| NLR | 3.01 ± 2.48 | 3.99 ± 2.91 | 0.048 |
| LCR | 7.69 ± 10.02 | 8.23 ± 9.34 | 0.031 |
| PLR | 135,667.73 ± 51,589 | 14,478.67 | 0.005 |
| **≥3 cardiovascular risk factors** | | | |
| Total cholesterol, mg/dL | 189.03 ± 36.78 | 190.36 ± 44.70 | 0.326 |
| LDL cholesterol, mg/dL | 121.03 ± 34.73 | 122.93 ± 38.87 | 0.157 |
| HDL cholesterol, mg/dL | 41.18 ± 10.57 | 41.90 ± 9.30 | 0.778 |
| Triglycerides, mg/dL | 134.09 ± 71.26 | 127.65 ± 51.23 | 0.239 |
| Serum creatinine, mg/dL | 1.09 ± 0.31 | 1.08 ± 0.36 | 0.537 |
| Serum urea, mg/dL | 45.64 ± 16.60 | 48.69 ± 22.67 | 0.098 |
| Fasting glucose, mg/dL | 142 ± 55.38 | 147.26 ± 55.54 | 0.099 |
| Serum fibrinogen, mg/dL | 411.74 ± 132.16 | 384.41 ± 120.66 | 0.173 |
| NLR | 3.75 ± 0.77 | 4.23 ± 1.01 | 0.019 |
| LCR | 7.86 ± 11.17 | 8.50 ± 11.89 | 0.007 |
| PLR | 141,889.12 ± 74,258.71 | 149,663.04 ± 76,752.19 | 0.042 |

**Table 3.** Correlations between inflammatory markers and demographic, anthropometric or clinical–paraclinical parameters.

| | Mild and Moderate Obstruction (*n* = 207) | | | | | | Severe Obstruction (*n* = 264) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NLR | | LCR | | PLR | | NLR | | LCR | | PLR | |
| | r | *p* | r | *p* | r | *p* | r | *p* | r | *p* | r | *p* |
| Total cholesterol | 0.085 | 0.0049 | 0.098 | 0.0033 | 0.025 | 0.0190 | 0.002 | 0.0268 | 0.022 | 0.0184 | 0.009 | 0.0240 |
| LDL cholesterol | 0.134 | 0.0009 | 0.151 | 0.0004 | 0.063 | 0.0088 | 0.572 | 0.0005 | 0.626 | 0.0012 | 0.715 | 0.0010 |
| HDL cholesterol | −0.027 | 0.0185 | −0.03 | 0.0177 | −0.076 | 0.0063 | −0.038 | 0.0125 | −0.055 | 0.0078 | −0.033 | 0.0141 |
| Triglycerides | −0.076 | 0.0063 | −0.078 | 0.0059 | −0.031 | 0.0171 | 0.023 | 0.0182 | 0.039 | 0.0122 | 0.011 | 0.0232 |
| Fasting glucose | 0.007 | 0.0252 | −0.043 | 0.0137 | −0.11 | 0.0240 | 0.416 | 0.0005 | 0.062 | 0.0061 | −0.014 | 0.0217 |
| Pulse pressure | −0.007 | 0.0253 | −0.017 | 0.0217 | −0.038 | 0.0153 | 0.029 | 0.0157 | 0.026 | 0.0168 | 0.008 | 0.0243 |
| Smoking—packs smoked per year | 0.113 | 0.0019 | 0.047 | 0.0125 | −0.009 | 0.0246 | 0.012 | 0.0226 | −0.014 | 0.0219 | 0.04 | 0.0259 |
| Number of risk factors | 0.317 | 0.0006 | 0.598 | 0.0017 | 0.921 | 0.0003 | 0.219 | 0.0004 | 0.468 | 0.0012 | 0.711 | 0.0007 |
| Pain at rest | 0.817 | 0.0013 | 0.643 | 0.0028 | 0.753 | 0.0009 | 0.416 | 0.0013 | 0.37 | 0.0014 | 0.446 | 0.0005 |
| Number of lesions | −0.77 | 0.0007 | −0.296 | 0.0018 | −0.538 | 0.0016 | 0.796 | 0.0005 | 0.234 | 0.0014 | 0.505 | 0.0004 |
| LVEF (%) | 0.065 | 0.0083 | 0.039 | 0.0148 | 0.426 | 0.0071 | −0.024 | 0.0178 | −0.015 | 0.0213 | −0.083 | 0.0028 |
| Risk of amputation | 0.158 | 0.0024 | 0.227 | 0.0023 | 0.301 | 0.0024 | 0.712 | 0.0005 | 0.331 | 0.0012 | 0.488 | 0.0010 |

r: Pearson's correlation; LDL: low-density lipoproteins; HDL: high-density lipoprotein; BMI: body mass index; NLR: neutrophil-to-lymphocyte ratio; LCR: lymphocyte-to-CRP ratio; PLR: platelet-to-lymphocyte ratio; LVEF: left ventricle ejection fraction.

*2.4. Ethics*

The study was approved by the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy Iasi and the Ethics Committee of "St. Spiridon" Clinical Emergency Hospital, and it was conducted according to the Helsinki Declaration. All

patients signed an informed consent statement, which mentioned that the results would be used for research purposes.

## 3. Results

In our study, we included 652 patients diagnosed with PAD (84.7% males, with a mean age of 66.46 $\pm$ 10.47 years old) who were evaluated in our clinic from an inflammatory point of view. According to the ankle–brachial index (ABI) value, we formed two study groups: patients with mild and moderate obstruction (judged as an ABI > 0.5) and patients with severe obstruction (with an ABI value below 0.5). We analyzed several demographic, hemodynamic, biochemical and imaging parameters, which are presented in Table 1.

In terms of demographic characteristics between the two groups, no significant differences in age (65.39 $\pm$ 11.06 vs. 67.18 $\pm$ 10.32, $p$ = 0.333), gender (male patients 84.44% vs. 84.81%, $p$ = 0.897) or residence (urban area 41.25% vs. 42.28%) were reported. In the case of anthropometric parameters, statistically significant differences were reported between the two groups analyzed, with patients with severe obstruction having higher mean weights (69.78 $\pm$ 9.66 vs. 81.20 $\pm$ 10.89, $p$ = 0.048) and BMI scores (25.10 $\pm$ 2.99 vs. 27.15 $\pm$ 3.16 kg/m$^2$, $p$ = 0.053) than patients in the first group.

Of the vital parameters assessed, pulse pressure (71.54 $\pm$ 11.73 vs. 75.81 $\pm$ 14.93 mmHg, $p$ < 0.001) was statistically significantly correlated with the degree of obstruction assessed using ABI. Patients with PAD enrolled in this study had a variety of comorbidities or cardiovascular risk factors. Cerebrovascular disease was more commonly present in patients with mild and moderate obstruction (8.95% vs. 7.09%, $p$ = 0.387), but it was not a statistically significant parameter.

Smoking, which is one of the main risk factors associated with the development and progression of atherosclerotic lesions in patients with PAD, was more frequently associated with the group of patients with mild and moderate obstruction (71.60% vs. 63.54%, $p$ = 0.033). Also, reporting the number of cigarettes smoked revealed a higher mean number of packs were smoked per year by patients with mild and moderate obstruction (25.63 $\pm$ 19.71 vs. 22.99 $\pm$ 18.94, $p$ = 0.043) than those with an ABI less than 0.5.

Dyslipidemia was present in more than 50% of patients in both groups ($p$ = 0.076), making it similar to dyslipidemia (hypercholesterolemia or hypertriglyceridemia, $p$ > 0.05), hypertension (50.58% vs. 46.84%, $p$ = 0.479) or class I obesity (67.7% vs. 73.2%, $p$ = 0.762).

Regarding the number of risk factors, the majority of patients in both groups had associated two cardiovascular risk factors (32.7% vs. 39.1%, $p$ = 0.041). Regarding lipid and carbohydrate profiles, no statistically significant differences were reported based on the severity of obstruction, as shown in Table 1.

Among the biological parameters, statistical analysis revealed statistically significant values for conventional and new inflammatory markers. The mean serum of high-sensitivity C-reactive protein (hs-CRP) (4.97 $\pm$ 3.01 mg/dL vs. 7.07 $\pm$ 3.83 mg/dL, $p$ = 0.023) and serum fibrinogen levels (369.47 $\pm$ 115.96 vs. 414.71 $\pm$ 137.97, $p$ = 0.001) were higher in patients with severe obstruction. The mean serum values of the inflammatory markers discussed in this paper were higher among patients with severe obstruction and considered to be statistically significant parameters in our group of patients ($p$ = 0.037 for NLR, $p$ = 0.041 for LCR and $p$ = 0.019 for PLR).

The number and severity of atherosclerotic lesions in the vascular axis of lower limbs were assessed and quantified using angiography. Peripheral angiography predominantly identified a stenotic lesion in both groups (37.74% vs. 32.65%). The percentage of patients with more than 6 stenotic lesions identified was higher in patients with severe obstruction (4.27% vs. 5.83%, $p$ < 0.001). Therapeutic management was performed in an integrative manner. In addition to drug treatment, a significant percentage of patients enrolled in the study received treatment via revascularization techniques. Interventional revascularization was preferred in patients with mild and moderate obstruction (12.06% vs. 4.30%, $p$ < 0.001), while a higher percentage of patients with severe obstruction benefited from surgical revascularization (51.36% vs. 59.75%, $p$ = 0.061). The risk of amputation was higher in

patients with ABI values below 0.5 (24.12% vs. 37.47%, $p < 0.001$). Taking into account the staging based on the WIfl classification, patients with severe obstructive lesions had a higher mean score than PAD patients with mild and moderate atherosclerotic lesions ($4.88 \pm 0.54$ vs. $5.37 \pm 0.61$, $p = 0.047$).

The main parameters of the lipid profile, carbohydrate profile and inflammatory markers were statistically analyzed based on the number of associated cardiovascular risk factors (Table 2). In patients with two associated risk factors, the serum fibrinogen level was found to be a statistically significant parameter, along with the evaluated inflammatory markers. In patients with more than three associated cardiovascular risk factors, patients with severe obstruction had higher mean serum values for NLR ($3.75 \pm 0.77$ vs. $4.23 \pm 1.01$, $p = 0.019$), PLR ($141,889 \pm 74,258.71$ vs. $149,663.04 \pm 76,752.19$, $p = 0.042$) and LCR ($7.86 \pm 11.17$ vs. $8.50 \pm 11.89$, $p = 0.007$), which were associated with a more pronounced inflammatory state.

Patients with gangrene are frequently associated with a high titer of inflammatory markers, which is why we decided to perform an analysis of the subgroup of patients without gangrene and with serum hs-CRP values below 10 mg/dL (first group—29 patients with gangrene and 21 patients with hs-CRP values above the mentioned limit; second group—92 patients with gangrene and 39 patients with hs-CRP values above 10 mg/dL; final analysis: 207 patients vs. 264 patients with PAD).

We identified several statistically significant correlations (after adjusting for various co-founders, such as age, anthropometric parameters or the presence of gangrene) in our study group, as shown in Table 3. Among the lipid profile parameters, LDL cholesterol had a direct proportional association with all three proposed biomarkers in patients with severe obstruction. NLR was statistically significantly correlated with the number of cardiovascular risk factors present in both patients with mild or moderate obstruction ($p = 0.0006$) and those with severe obstruction ($p = 0.0004$). The number of angiographically detected atherosclerotic lesions was also statistically significantly correlated with NLR in patients included in the first group ($p = 0.0007$), as well as with NLR ($p = 0.0005$). LCR ($p = 0.0014$) and PLR ($p = 0.0004$) were statistically significantly correlated with patients in the second group. The risk of amputation was assessed in all patients enrolled in this study, with statistically significant correlations noted between its presence and NLR ($p = 0.0005$), LCR ($p = 0.0012$) and PLR ($p = 0.0010$) in the group of patients with ABI values below 0.5 (Figures 2 and 3). The predictive value of NLR and PLR was also demonstrated using univariate and multivariate statistical analysis, as shown in Table 4.

**Table 4.** Univariate and multivariate statistical analysis for NLR, PLR and LCR among patients with severe obstruction.

| Parameter | Univariate Regression | | | Multivariate Regression | | |
|---|---|---|---|---|---|---|
| | $\beta$ | $p$ | Odds Ratio (95% CI) | $\beta$ | $p$ | Odds Ratio (95% CI) |
| LCR | 0.043 | 0.015 | 1.051 (1.009–1.085) | | | |
| NLR | 0.179 | <0.001 | 1.292 (1.131–1.290) | 0.025 | 0.029 | 1.054 (1.005–1.105) |
| PLR | 0.033 | 0.002 | 1.053 (1.011–1.044) | 0.525 | 0.005 | 0.591 (0.410–0.852) |

We evaluated the weights of cardiovascular risk factors in the two groups of patients and observed that the majority of patients presented two risk factors (24.58% vs. 26.63%, $p = 0.194$ for the first group and $p = 0.804$ for the second group). Patients with mild and moderate obstruction, as well as those with ABI values below 0.5, showed statistically significant correlations between the risk of amputation and the presence of gangrene or intermittent claudication at rest ($p < 0.001$ for all associations) (Figure 4).
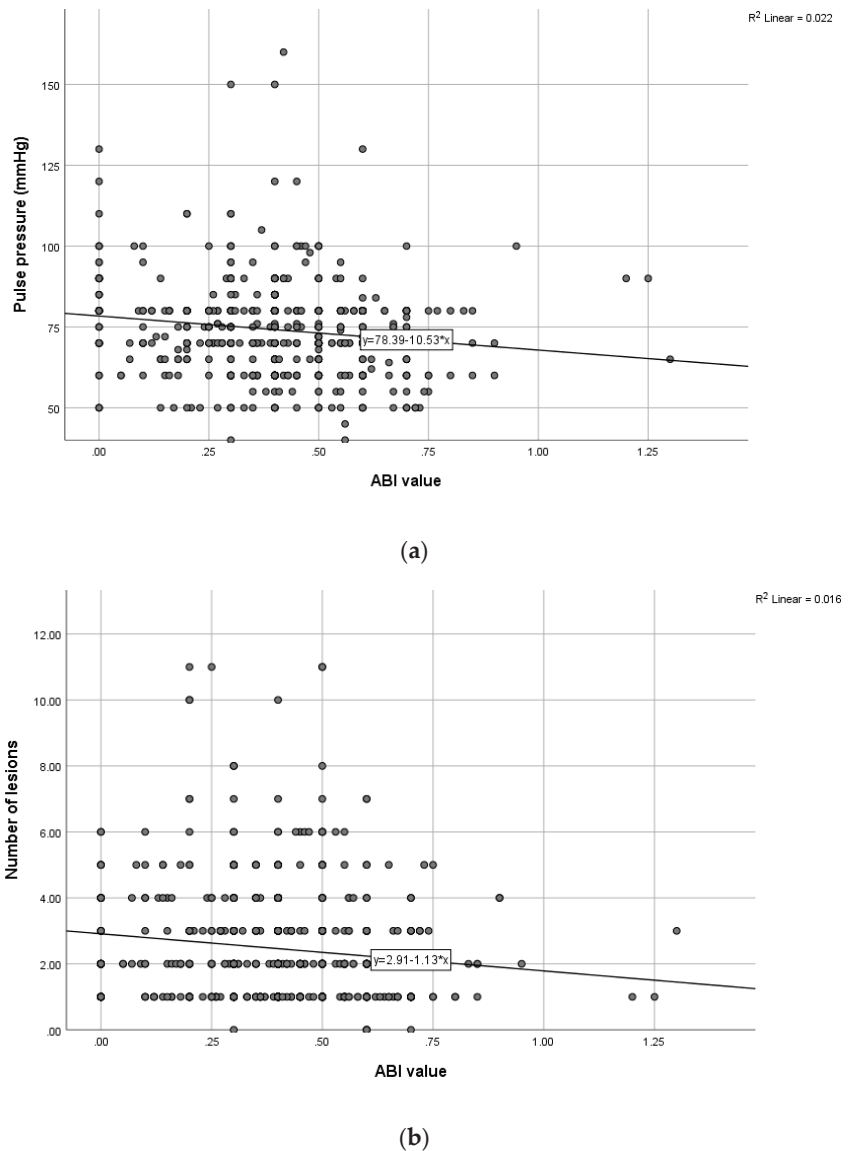
(**a**)



(**b**)

**Figure 2.** Correlation between ABI and pulse pressure (**a**) or the number of lesions (**b**) (ABI: ankle–brachial index).

The respective values of NLR, LCR and PLR predictors associated with amputation risk in patients with severe obstruction were via receiver operating characteristic (ROC) analysis (Figure 5). NLR (area under the curve <AUC> = 0.682, $p$ = 0.010, 95% confidence interval <CI> 0.419–0.664) and PLR (AUC = 0.692, $p$ = 0.006, 95% CI 0.556–0.829) are inflammatory markers associated with a high risk of amputation, while LCR (AUC = 0.541, $p$ = 0.558, 95% CI 0.419–0.664) did not prove its value as a predictor in our study. In addition to the markers presented above, we tested the predictive value associated with amputation risk for hs-CRP, with this classic marker being a statistically significant predictor in our study group, as shown in Figure 6.
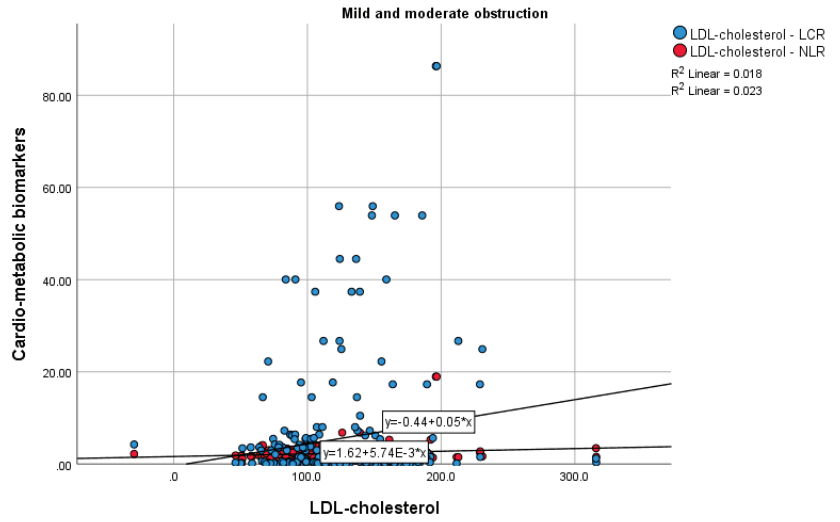
**Figure 3.** Correlation between LDL cholesterol, NLR and LCR in patients with mild and moderate obstruction. (LDL: low-density lipoproteins NLR: neutrophil-to-lymphocyte ratio; LCR: lymphocyte-to-C-reactive protein ratio).



(**a**)
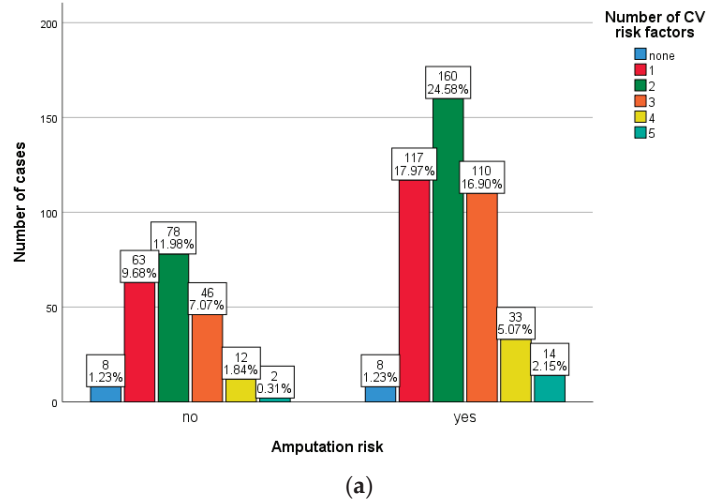
**Figure 4.** *Cont.*
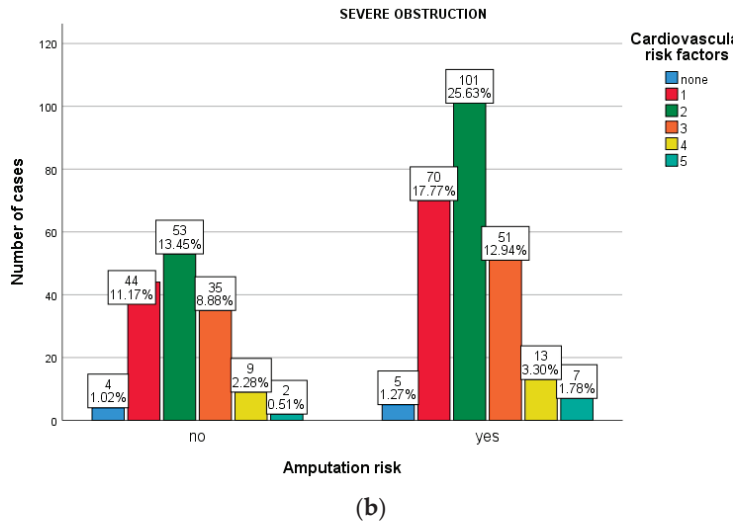
**Figure 4.** Weight of cardiovascular risk factors according to the risk of amputation in patients with mild and moderate (**a**) or severe obstruction (**b**).
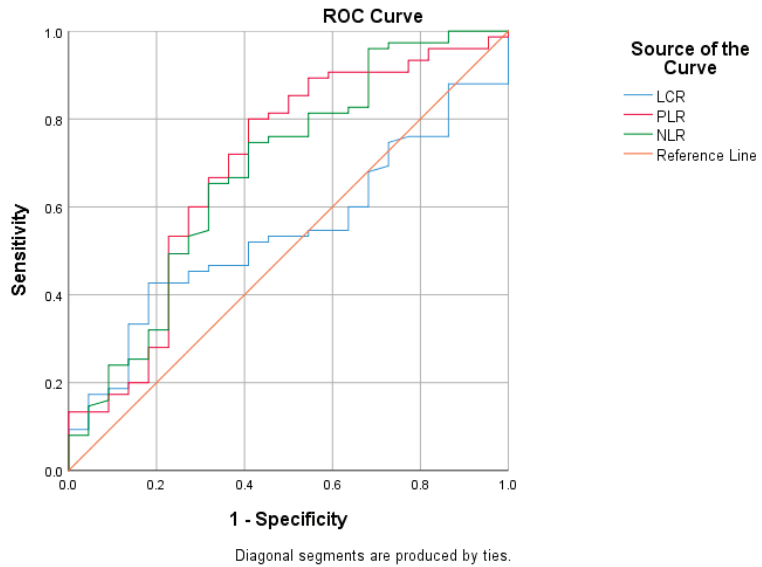


Diagonal segments are produced by ties.

**Figure 5.** The area under the curve of the receiver operating characteristic used to determine inflammatory biomarkers in patients with amputation risk and severe obstruction (AUC: area under the curve, NLR: neutrophil-to-lymphocyte ratio; LCR: lymphocyte-to-C-reactive protein ratio, PLR: platelet-to-lymphocyte ratio).
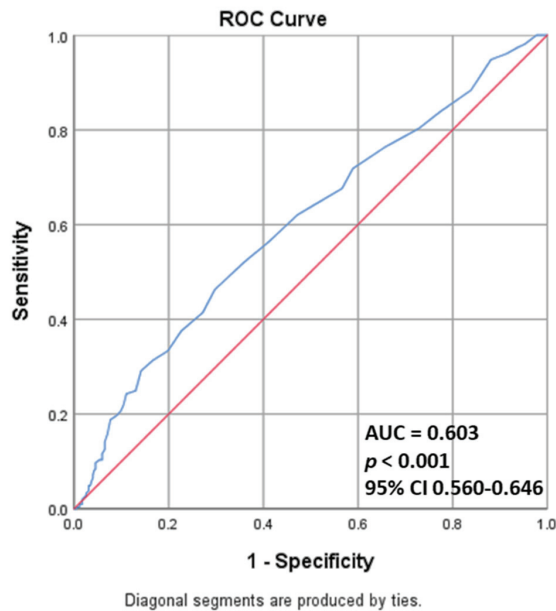
**Figure 6.** The area under the curve of the receiver operating characteristic hs-CRP in patients with amputation risk and severe obstruction (AUC: area under the curve; CI: confidence interval).

## 4. Discussion

Atherosclerosis is a multifactorial process in which inflammatory status plays a role in determining both the appearance of lesions and their progression and, therefore, in increasing the risk of an acute cardiovascular event [28,29]. In total, 84.7% of the patients enrolled in our study were males, with a mean age of $66.46 \pm 10.47$ years. By analyzing a broad spectrum of parameters, we highlighted the role played by the proposed inflammatory biomarkers (NLR, PLR, LCR) in the management of PAD and their prognostic implications.

Various degrees of inflammation have been identified at all stages of PAD, with this association also being thoroughly researched. Some studies indicate a stronger association with PAD than with coronary artery disease, suggesting the presence of different predominant substrates [30]. In our study, serum hs-CRP levels were elevated, regardless of the severity of obstruction, representing a statistically significant inflammatory marker ($p = 0.023$). Also, regarding medium- and long-term evolution of hs-CRP, it has been shown that in patients with PAD, high levels at the time of the first revascularization intervention and their persistence at 3.6 years of follow-up are associated with an independent increase in all-cause, cardiovascular and malignancy-related mortality, with these results being supported by other similar research [31,32].

The mean serum values of NLR ($p = 0.037$), LCR ($p = 0.041$) and PLR ($p = 0.019$) were higher in patients with severe obstruction, as well as statistically significant biomarkers in our analyzed group. Similar results have been reported by other investigators in the literature, with the calculation of these biological parameters having both therapeutic and prognostic value.

The role played by NLR as a predictive factor in assessing the risk of death or an acute cardiovascular event has been extensively reviewed in the literature [33–35], and the reported results are similar to those obtained in this study. Positive and statistically significant correlations were found between NLR, LDL cholesterol, fasting glucose and the number of cardiovascular risk factors, as well as the presence of gangrene and the number of atherosclerotic lesions angiographically identified. A meta-analysis including 38 clinical studies summarizing 76,000 patients demonstrated that high NLR values increased the risk

of coronary artery disease (CAD) 1.62-fold and the risk of stroke 3.86-fold, which justifies regular evaluation of the complete blood count [36].

Patients with PAD are associated with a high risk of developing an acute vascular event in the absence of integrative management [37]. A group of Romanian researchers demonstrated that high NLR and PLR values are associated with an 11-fold increase in the risk of amputation and a 22-fold increase in the risk of death in patients with acute limb ischemia [8]. Similar results were reported by Coelho et al. [38], who analyzed a group of 345 patients with acute inferior ischemia and demonstrated that an NLR value above 5.4 is consistent with a sensitivity of 90.5% and specificity of 73.6% for the occurrence of death at 30 days or amputation. PAD patients with obstructive atherosclerotic lesions at the femuro-popliteal level who undergo peripheral revascularization surgery and have associated high pre-operative NLR and PLR values have an increased risk of primary patency failure at 12 months after revascularization [39]. Erturk et al. [40] analyzed a group of 593 patients with occlusive PAD and divided them into two groups according to the NLR value (below 3 and above 3), observing that age and NLR values above three are independent factors associated with long-term mortality in these patients.

Cosarca et al. [7] demonstrated that NLR values above 3.48 have a sensitivity of 60% and a specificity of 72.44% regarding the need for amputation after revascularization in patients with PAD, thus making them a useful pre-operative prognostic marker. Similarly, the same group of investigators demonstrated in PLR that serum values above 152 are associated with a sensitivity of 54.17% and a specificity of 71.79% regarding amputation. Increased absolute neutrophil counts relative to lymphocyte counts are associated with a poorer prognosis in PAD patients undergoing interventional revascularization [41,42].

Similar to the NLR, the PLR has a predictive value regarding the risk of an acute vascular event; in the case of patients with PAD, the existence of a value of more than 150 is associated with a relative risk about two times higher than that of critical atherosclerotic lesions [43]. Liu et al. [44] analyzed a cohort of 355 diabetic patients, in whom they assessed the risk of developing PAD and identified NLR and PLR as predictors associated with the development and progression of atherosclerotic processes in this category of patients, finding evidence of the superiority of PLR.

The validity of PLR's use as an inflammatory marker is secondary to the pro-inflammatory effect exerted by platelets [45]. Initially investigated in various oncological clinical trials [46], this biomarker has increasingly broad validity as a predictor of moderate-to-severe functional decline in PAD patients, as demonstrated above. PLR is another biomarker that plays a prognostic role in the management of patients with PAD, with elevated titers being associated with a high risk of critical ischemia or acute vascular events (odds ratio of 1.9 for PLR > 150) [43].

PLR also modulates the risk of death among patients with PAD. Uzun et al. [47] demonstrated through the analysis of a cohort of 602 patients with PAD that the identification of a PLR value above 142 is an independent predictor of an increased long-term risk of death.

In addition to the biomarkers mentioned above, the monocyte-to-HDL cholesterol ratio was analyzed in relation to the severity and prognosis of PAD, but the reported results have so far been contradictory [48]. Clinical studies reported in the literature that do not report superior results for this inflammatory marker compared to NLR also exist [48]. On the other hand, Selvaggio et al. [10] reported the existence of a directly proportional relationship between increases in PLR and the monocyte-to-HDL cholesterol ratio and decrease in ABI ($p = 0.0011$). Guetl et al. [49] conducted a retrospective study in which 2121 patients with PAD were included and, using multivariate regression statistical analysis, demonstrated that increased WMR values (odds ratio 2.25, $p < 0.001$), older age (odds ratio 1.05, $p < 0.001$), elevated CRP titer (odds ratio 1.01, $p < 0.001$) and diabetes mellitus (odds ratio 2.38, $p < 0.001$) were independently significant predictors of chronic limb-threatening ischemia occurrence.

Gary et al. [50] demonstrated that patients with NLR values above 3.95 have a 2.5-fold increased risk of critical lower limb injury, making this inflammatory biomarker an easily measured prognostic parameter that can be used in everyday practice. Neutrophilia is responsible for increasing the value of the ratio, being the result of various pathophysiological processes that contribute to the maintenance of the pro-inflammatory status in PAD [51]. Taşoğlu et al. [52] showed that the presence of an NLR value above 3.2 and a PLR above 160 are associated with a high risk of amputation, with the average duration being about 2 years to date.

A significant percentage of patients with associated PAD and CAD had this condition as an issue secondary to existing atherosclerotic damage, which was sometimes subclinical in nature. In this category of patients, Arbel et al. [53] demonstrated that an NLR value above three is associated with a relative risk of 2.45 regarding the existence of sub-occlusive coronary lesions, as well as the occurrence of an acute cardiovascular event in the next 3 years (odds ratio: 1.55). Yuan et al. [54] analyzed a cohort of 235 patients with COPD and demonstrated a positive correlation between NLR and WBC, hs-CRP, BMI and 6-min walking test distance, thus making it an indicator of muscle function in this category of patients. Interruption of regular physical training also produced a number of negative changes in inflammatory parameters, with a 48.2% increase in NLR reported in the clinical study by Liao et al. [55].

Our study presents several limitations due to the lack of follow-up. The heterogeneity of the study group or the potential risk associated with the inclusion of patients with elevated serum CRP values due to associated infections are additional aspects that may influence the obtained results. We excluded records in which medical data were unavailable. This step was taken to minimize the risk of misclassification, introducing a limited risk of selection bias.

Our future research direction will be to investigate the influence of the proposed markers (NLR, PLR, CSF) on the predictive value of amputation risk in relation to a series of biochemical or clinical models, such as PREVENT III or the BASIL model, that exist in the literature [56].

## 5. Conclusions

In our study, we demonstrated the predictive value of the analyzed inflammatory biomarkers and the importance of their assessment in patients with severe obstruction and a high risk of amputation. NLR and PLR are predictors used in patients with ABI values below 0.5 and a risk of amputation, thus making them parameters with both therapeutic and prognostic value. NLR, PLR and WMR are easy-to-determine and reproducible parameters, which can be easily used in daily practice, as they also have therapeutic and prognostic value among patients with PAD.

**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy Iași and the Ethics Committee of "St. Spiridon" Clinical Emergency Hospital.

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** All the data are available from the corresponding author upon reasonable request.

**Conflicts of Interest:** The authors declare no conflict of interest.

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*Systematic Review*

# Effectiveness and Safety of Fufang Danshen Dripping Pill (Cardiotonic Pill) on Blood Viscosity and Hemorheological Factors for Cardiovascular Event Prevention in Patients with Type 2 Diabetes Mellitus: Systematic Review and Meta-Analysis

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**Abstract:** *Background and Objectives*: Diabetes can cause various vascular complications. The Compounded Danshen-Dripping-Pill (CDDP) is widely used in China. This study aimed to analyze the effectiveness and safety of CDDP in the blood viscosity (BV) with type 2 diabetes mellitus (T2DM). *Materials and Methods*: We conducted a systematic search of seven databases from their inception to July 2022 for randomized controlled trials that used CDDP to treat T2DM. To evaluate BV, we measured low shear rate (LSR), high shear rate (HSR), and plasma viscosity (PV). Homocysteine and adiponectin levels were also assessed as factors that could affect BV. *Results*: We included 18 studies and 1532 patients with T2DM. Meta-analysis revealed that CDDP significantly reduced LSR (mean difference [MD] −2.74, 95% confidence interval [CI] −3.77 to −1.72), HSR (MD −0.86, 95% CI −1.08 to −0.63), and PV (MD −0.37, 95% CI −0.54 to −0.19) compared to controls. CDDP also reduced homocysteine (MD −8.32, 95% CI −9.05 to −7.58), and increased plasma adiponectin (MD 2.72, 95% CI 2.13 to 3.32). Adverse events were reported less frequently in the treatment groups than in controls. *Conclusions*: CDDP is effective in reducing BV on T2DM. However, due to the poor design and quality of the included studies, high-quality, well-designed studies are required in the future.

**Keywords:** systematic review; meta-analysis; type 2 diabetes mellitus; Fufang danshen dripping pill; blood viscosity

## 1. Introduction

Diabetes mellitus is a metabolic disease characterized by insulin resistance in the target organs or an absolute or relative insulin deficiency and can be classified into type 1 diabetes mellitus, type 2 diabetes mellitus (T2DM), and gestational diabetes [1]. Diabetes is a major cause of numerous micro- and macrovascular complications that not only reduce the

quality of life and life expectancy, but can also lead to blindness, kidney failure, myocardial infarction, stroke, and limb amputation [2]. The prevalence of diabetes worldwide is increasing, and the International Diabetes Federation (IDF) estimates that the number of people with diabetes will reach 783.2 million in 2045 and the number of people with diabetes will increase by 46% by 2045, which is expected to reach 783.2 million people [3–5]. Currently, the global prevalence is estimated to be over 10.5%, and the increase in prevalence is particularly rapid in Asia. The increase in the diabetic population is associated with changes in lifestyle factors, including increased prevalence of overweight and obesity, social factors such as smoking, westernized diets, and changes to sedentary lifestyles [6]. As the number of patients with diabetes increases, there is increasing pressure on the national health system to treat diabetes and diabetic complications; therefore, prevention and early treatment of diabetes are important. As the prevalence of T2DM increases, so does the risk of cardiovascular disease [7–9]. Adults with diabetes are known to have a 2–4 times increased cardiovascular risk compared with adults without diabetes [10]. The ultimate goal of diabetes mellitus management is to prevent various complications and lower the mortality rate. Conventional therapies aiming for this primarily include the drugs Metformin, DDP-4 inhibitor, SGLT2-inhibitor, Sulfonylureas, and Thiazolidinediones. If blood sugar levels are not controlled with these oral medications, insulin injection is sometimes used [11,12]. However, these conventional medicines have a number of side effects: there have been cases of hypoglycemia with the use of sulfonylurea, there is a report that an alpha-glucosidase inhibitor causes liver dysfunction, and acute pancreatitis or joint dysfunction has been reported with the use of DDP-4 [13,14]. Therefore, a complementary therapeutic option is needed for use with existing hyperglycemic agents when blood sugar levels are controlled poorly or to reduce the occurrence of diabetic complications [15].

Oxidative stress plays an important role in cardiovascular disease in diabetic patients [16]. Among blood rheological properties, blood viscosity is known to be a major predictor of oxidative stress, previous studies have shown that blood viscosity is elevated in people with high oxidative stress, such as lead-exposed laborers or smokers, compared to healthy individuals [17,18]. In addition, it is known that blood viscosity is higher in patients with type 2 diabetes than in healthy people [19,20]. Therefore, effective management of blood viscosity is required to prevent cardiovascular disease in patients with T2DM; however, no drugs are known to be effective against blood viscosity.

In East Asia, various herbal medicines and acupuncture are being used to treat diabetes and its associated complications [21,22]. Among these herbal prescriptions, the Compounded Danshen Dripping Pill (CDDP; also known as the cardiotonic pill) is a drug used for coronary vascular disease management in China and has been reported to be effective in lowering blood sugar and treating diabetic complications, including blood viscosity (Figure 1). In particular, CDDP has been shown to suppress oxidative stress and inflammatory responses in animal experiments [23,24]. Clinical studies have also reported that CDDP is effective in reducing triglyceride or low-density lipoprotein cholesterol (LDL-C) levels and slowing the progression of diabetic retinopathy when used in combination with aspirin in a coronary disease case [25,26]. However, there is no comprehensive review on the effect of CDDP on blood oxidative stress in diabetic patients, and there is no study on the effect of CDDP on blood viscosity and related hemorheological indices. Therefore, this study aimed to analyze the effectiveness and safety of CDDP in T2DM via a systematic review to summarize the evidence and suggest implications for further study and clinical practice.
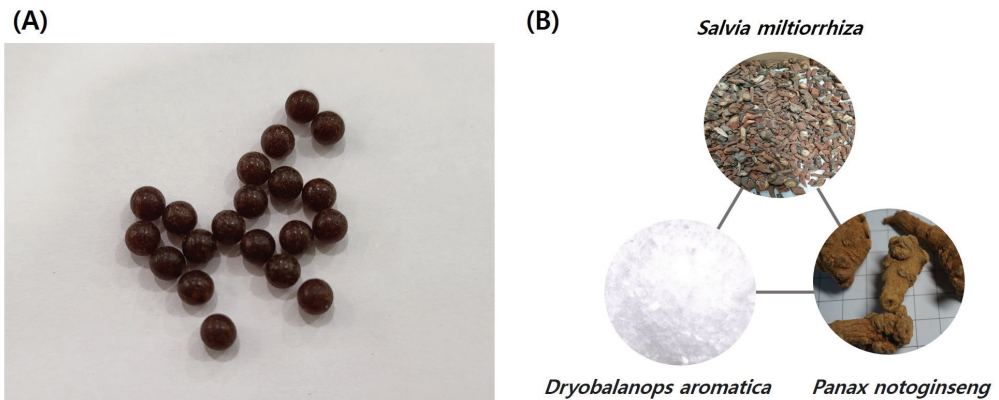
**Figure 1.** Compounded Danshen Dripping Pills (CDDPs). (**A**) CDDPs are a brown colored, round pill, usually 4.5 mm (0.18 inch) in size. (**B**) The three main herbal components of CDDP are *Salvia miltiorrhiza*, *Dryobalanops aromatica*, and *Panax notoginseng*.

## 2. Materials and Methods

### 2.1. Study Registration

The systematic literature review protocol was prepared according to the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) guidelines. The systematic review protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO; Registration ID: CRD42022352381). Since this study quantitatively synthesized data from previously published papers, institutional review board approval and participant consent were not required.

Searching Strategy

The following seven databases were searched from their inception to September 2022: MEDLINE (PubMed, https://pubmed.ncbi.nlm.nih.gov/ accessed on 30 September 2022), Cochrane Library (CENTRAL, https://www.cochranelibrary.com/), EMBASE (https://www.embase.com), OASIS (https://oasis.kiom.re.kr/), Korea Citation Index (KCI; https://www.kci.go.kr), and China National Knowledge Infrastructure (CNKI; https://oversea.cnki.net/index/), Research Information Sharing Service (RISS; http://www.riss.kr/index.do). The search strategies are shown in Supplementary S1.

### 2.2. Eligibility Criteria for Study Selection

2.2.1. Types of Studies

The systematic review and meta-analysis included published peer-reviewed randomized controlled trials (RCTs). We manually searched for studies of patients with type 2 diabetes who were treated with CDDP and checked blood viscosity for potential inclusion.

2.2.2. Types of Participants

Eligible participants were patients diagnosed with T2DM. There were no restrictions based on sex, ethnicity, symptom severity, disease duration, or clinical environment.

2.2.3. Types of Interventions and Comparators

The research question of this review was 'Does CDDP have a significant effect on blood viscosity, homocysteine, and plasma adiponectin levels in T2DM patients?' The definition of CDDP was limited to the 'Fufang danshen dripping pill' of Tasly Pharm Co., Tianjin, China. Other names for CDDP, such as 'cardiotonic pills' and 'Fufang danshen dripping pill,' were included in the search strategy. The control group was defined as conventional Western medicine therapy for diabetes and included studies that used a placebo group

administered a herbal medicine that had the same appearance and flavor as CDDP. The included studies were limited to oral medications, excluding other routes of administration such as injections.

### 2.2.4. Types of Outcome Measures

#### Primary Treatment Outcome: Final Value of Blood Viscosity (Low and High Shear Rate)

For the confirmation of blood viscosity, the cone plate rotation and scanning capillary methods were considered representative. The cone plate rotation method is a technique that measures the blood resistance when a certain amount of blood is put between the cone and the plate and rotated, and converts it into blood viscosity [27]. In the case of the scanning capillary method, the blood is passed through an Ethylenediaminetetraacetic acid (EDTA) tube and the viscosity of the blood is measured. In the cone plate rotation method, high blood viscosity (known as systolic blood viscosity or high shear rate; HSR) ($300 \ s^{-1}$) was 3.25–4.91 for men and 2.94–4.59 for women, and low blood viscosity (known as diastolic blood viscosity or low shear rate; LSR) ($5 \ s^{-1}$) was 7.75–11.48 for men and 7.23–10.61 for women [28]. In the scanning capillary method, the HSR was 3.5–4.1 in men and 3.0–3.6 in women, and the LSR was 9.35–13.1 in men and 7.59–11.13 in women [29]. Factors affecting blood viscosity include not only blood glucose, but also fibrinogen, red blood cells (RBCs), white blood cells (WBCs), and triglyceride levels [17,30,31]. A recent study showed that blood viscosity at a low shear rate was associated with the occurrence of early neurological deterioration in patients with lacunar infarction [32]. Furthermore, hyper-viscosity can occur with COVID-19 infection, causing poor tissue perfusion, peripheral vascular resistance, and thrombosis [33].

#### Secondary Treatment Outcomes: Plasma Viscosity, Homocysteine, and Plasma Adiponectin Levels

Plasma viscosity: The quantity of proteins in the blood has an impact on the viscosity of plasma [34]. The normal plasma viscosity range is 1.10–1.30 mPas at 37.7 °C [35].

Homocysteine: Homocysteine is a sulfur-containing amino acid and is generated from the breakdown of the dietary amino acid methionine [36]. Excessive accumulation of homocysteine can increase the risk of brain infarction or dementia and is a risk factor for atherosclerosis [37,38]. Furthermore, higher homocysteine levels were observed in T2DM patients than in controls, indicating that a higher homocysteine level is a novel risk factor for predicting diabetic complications such as diabetic retinopathy [39]. Since homocysteine is a type of amino acid associated with hyper-viscosity, along with multiple other factors related to blood viscosity, it was investigated as a secondary outcome [40].

Adiponectin: Adiponectin, also known as adipocyte complement-related protein of 30 kDa (Acrp30) or AdipoQ, is a 244-amino acid protein secreted mainly by adipose tissue. Adiponectin is a hormone that initiates the use of body fat for energy, which is found primarily in white fat tissue and less in brown fat tissue [41]. People with higher levels of adiponectin are less likely to develop cardiovascular disease than those with low levels [42]. Moreover, some studies reported that higher levels of adiponectin are associated with a lower risk of high blood pressure, cardiovascular disease, and obesity [43–45]. Circulating adiponectin levels have been correlated with factors affecting blood viscosity, such as RBC deformability and systemic inflammatory markers [46,47]. Thus, this study investigated adiponectin as a secondary outcome.

#### Primary Safety Outcome: Rate of Adverse Events

To measure the adverse events rate, the study verified adverse events reported in the included clinical trials and compared the adverse events rate between the control group and the CDDP group.

### 2.2.5. Data Extraction and Risk of Bias Assessment

Two independent authors (MY and YK) performed data extraction and quality assessment of the RCTs using a data extraction form and Excel software (version 2201). The form included the year of study publication, participant characteristics, sample size, duration of treatment, frequency of treatment, type of comparator, type of outcomes, and adverse events.

The risk of bias was assessed using the tool from the Cochrane Handbook Version 6.0, which included random sequence generation, allocation concealment, blinding of the participants and personnel, blinding of the outcome assessments, incomplete outcome data, selective reporting, and other sources of bias [48]. Random sequence generation was evaluated as 'low risk' when the random sequence of the study was described in detail, and as 'unclear risk' if no other specific methodology was mentioned. In allocation concealment, studies in which it was unknown whether a third researcher was assigned were evaluated as 'unclear risk'. In blinding of participants and personnel, studies prescribing CDDP as an add-on medicine in the treatment group were evaluated as 'high risk' because the patient and researcher could have known which group the subject belonged to, and studies using placebo were evaluated as 'low risk'. Blinding outcome assessment was generally evaluated as 'low risk' because it was an objective blood test result, such as blood viscosity, homocysteine, and plasma adiponectin. In incomplete outcome data, studies with no missing values or that indicated the reason for dropout were evaluated as 'low risk', and studies that did not indicate the reason for the patient's dropout were evaluated as 'unclear risk'. In selective reporting, publications where the study protocol could not be found were evaluated as 'unclear risk'; all included studies were evaluated as 'unclear risk' because the protocol was not searched. In other sources of bias, studies were considered 'low risk' when there were no factors that could affect the research results, such as conflicting results with research from other sources (following research quality evaluation) or when the research was sponsored by a specific company. Two authors independently verified the outcomes of this procedure and any discrepancies were resolved through discussion.

### 2.2.6. Data Synthesis

For the quantitative synthesis of continuous variables, we calculated the mean difference (MD) and 95% confidence interval (CI) for the final values. Heterogeneity was assessed using the I2 statistic, and a random-effects model was used to account for any observed heterogeneity. Publication bias was tested using funnel plots and Egger's test [49].

We assessed the overall quality of the evidence using the GRADE approach. The quality of evidence was rated as high, moderate, low, or very low based on the study design, risk of bias, indirectness, imprecision, inconsistency, and publication bias. Generally, we rated the quality of evidence as moderate owing to some heterogeneity observed in the data synthesis and the possibility of publication bias [50].

## 3. Results

### 3.1. Study Selection

A total of 2649 articles were initially identified from six electronic databases. After excluding duplications, irrelevant studies, and review articles, 173 potentially eligible articles were selected. Finally, 18 studies including 1532 patients with T2DM were included (Figure 2 and Supplementary S2).
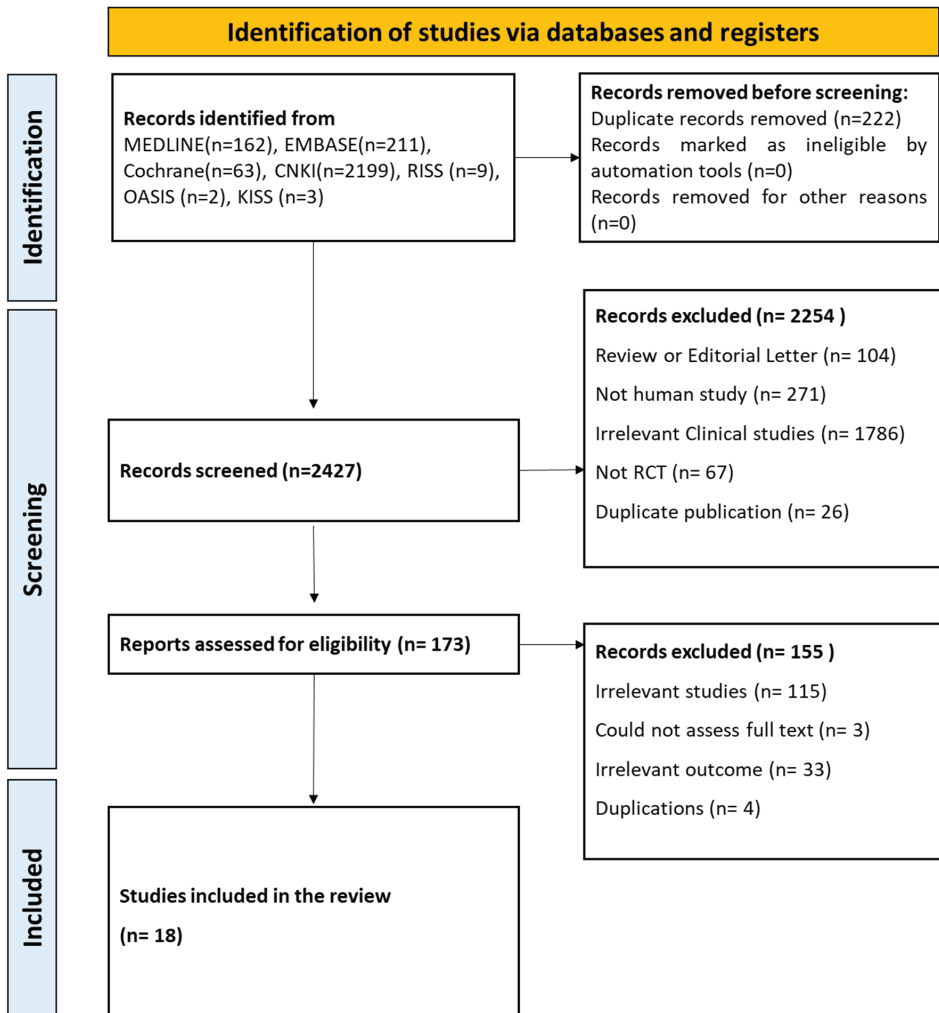
## Identification of studies via databases and registers

**Identification**

**Records identified from**
MEDLINE(n=162), EMBASE(n=211), Cochrane(n=63), CNKI(n=2199), RISS (n=9), OASIS (n=2), KISS (n=3)

**Records removed before screening:**
Duplicate records removed (n=222)
Records marked as ineligible by automation tools (n=0)
Records removed for other reasons (n=0)

**Screening**

**Records screened (n=2427)**

**Records excluded (n= 2254 )**
Review or Editorial Letter (n= 104)
Not human study (n= 271)
Irrelevant Clinical studies (n= 1786)
Not RCT (n= 67)
Duplicate publication (n= 26)

**Reports assessed for eligibility (n= 173)**

**Records excluded (n= 155 )**
Irrelevant studies (n= 115)
Could not assess full text (n= 3)
Irrelevant outcome (n= 33)
Duplications (n= 4)

**Included**

**Studies included in the review**
**(n= 18)**

**Figure 2.** Flowchart of this study.

### 3.2. Study Characteristics

The included studies were conducted in China and were published between 2005 and 2020. All studies were conducted on patients with diabetes. In particular, when classified by disease including duplicates, eight studies included patients with myocardial ischemia as a diabetic complication [51–58], one with diabetic retinopathy (with nephropathy) [59], six with diabetic nephropathy [59–64], and two with diabetic neuropathy [65,66]. Others were related to type 2 diabetes (Supplementary S3).

According to the intervention method, when the included clinical trials were classified, ten studies compared the effects between conventional Western medicine therapies and CDDP (head-to-head design) [51,53–58,65–67], and seven studies compared the effects between combined CDDP and Western medicine therapy and single Western medicine therapy [52,59,61–64,68]. Among them, two studies included dietary control in the treatment and control groups [59,60]. The final study compared the effects between combined CDDP and Western medicine therapy and combined placebo CDDP and Western medicine therapy [60].

*3.3. Quality of Evidence and Publication Bias*

To assess the risk of bias in our meta-analysis, we evaluated the included studies based on key criteria, including random sequence generation, allocation concealment, blinding of participants, blinding of outcome assessment, incomplete outcome data, and selective reporting. Our assessment revealed that 2/18 studies had a low risk of bias for random sequence generation [53,66]. The high number of unclear risk studies was due to the lack of clear information provided regarding the methods used for randomization. For allocation concealment, only one study clearly reported the method used and was assessed as having a low risk of bias [64]. With regards to the blinding of participants, only one study was assessed as having a low risk of bias, while the rest were assessed as having a high risk [60]. This was because the intervention and control groups received different interventions, making it impossible to blind the participants. Blinding of the outcome assessment was evaluated as having a low risk of bias for all studies included in our analysis. As the outcome of interest was a blood test, it was impossible for the participants or researchers to influence the outcome. In incomplete outcome data assessment, we found four studies had an unclear risk of bias for incomplete outcome data [51,54,64,68]. Finally, selective reporting was assessed as having a low risk of bias for all studies included in our analysis. This was because the number of participants in the intervention and control groups was reported before and after the intervention, ensuring that there was no selective reporting (Figures 3 and 4).
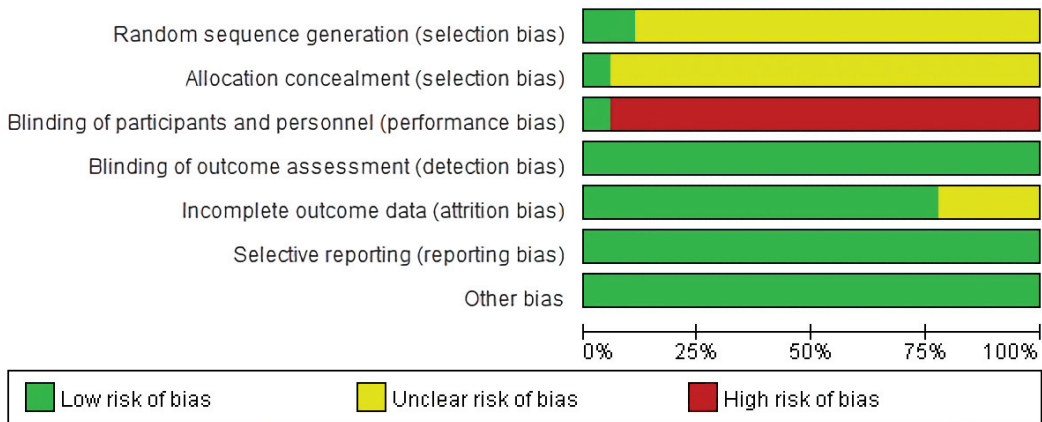


**Figure 3.** Risk of bias assessment.

*3.4. Effectiveness of CDDP on Blood Viscosity*

Studies were classified into subgroups according to the study design: (1) CDDP + Western Medicine (WM) (treatment group) vs. WM alone (control group), (2) CDDP alone (treatment group) vs. WM alone (control group), and (3) CDDP + WM (treatment group) vs. Placebo CDDP + WM (control group). For evaluating the effectiveness of CDDP on blood viscosity, five variables were measured: LSR, HSR, and plasma viscosity were measured as primary outcomes and homocysteine and adiponectin were measured as related factors. Publication bias according to individual variables is shown in Supplementary S4. The results of the GRADE assessment are shown in Supplementary S5.

**Figure 4.** Risk of bias summary for the 18 studies analyzed. Green '+' circles = Low risk of Bias; yellow '?' circles = uncertainties about the risk of bias; red '−' circles = high risk of bias. Reference: Bai 2008 [64]; Chen 2018 [53]; Gu 2020 [65]; Guo 2007 [60]; Huang 2005 [68]; Jia 2017 [63]; Jin 2015 [54]; Li 2015 [56]; Lin 2017 [66]; Lu 2017 [55]; Wang 2015 [58]; Wang 2016 [57]; Yang 2019 [62]; Ye 2016 [51]; Yin 2017 [67]; Yuan 2013 [61]; Zhang 2009 [59]; Zhao 2016 [52].

### 3.4.1. Low Shear Rate

When compared with controls, LSR was significantly lower in the CDDP group (7 studies, MD −2.74, 95% CI −3.77 to −1.72) (Figure 5A) [59–61,64,66–68]. LSR was then analyzed in the individual subgroups according to the study design. In the four studies using CDDP + WM vs. WM, LSR was significantly lower in the CDDP + WM group than in the WM group (MD −2.97, 95% CI −3.76 to −2.19) [59,61,64,68]. In the two studies using CDDP vs. WM, LSR was also significantly lower in the CDDP group than in the WM group (MD −3.17, 95% CI −3.91 to −3.50) [66,67]. In the one study using CDDP + WM vs. Placebo CDDP + WM, there was no significant difference between the groups (MD −0.28, 95% CI −0.98 to 0.42) [60].
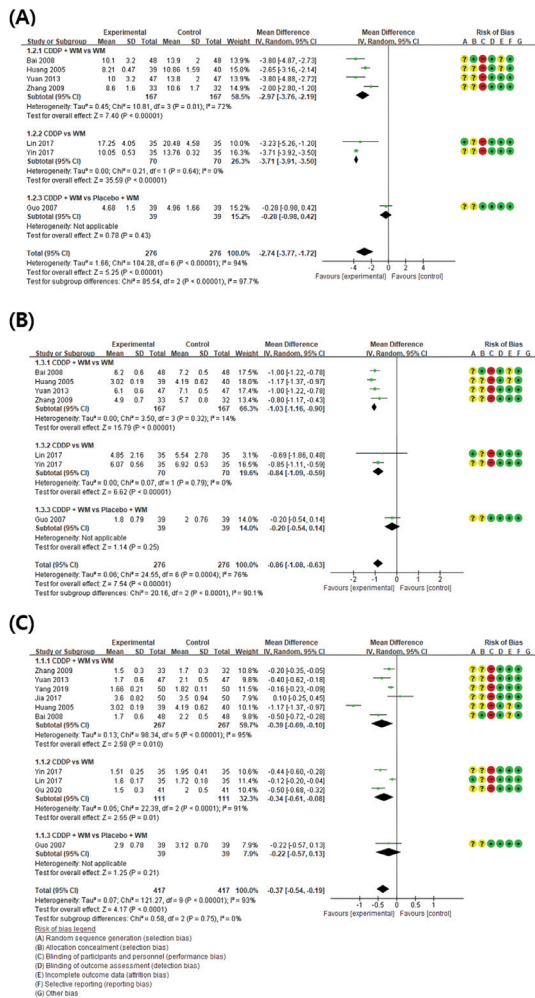


**Figure 5.** Meta-analysis of blood viscosity factors between treatment groups and control groups. (**A**) Low shear rate, (**B**) high shear rate, and (**C**) plasma viscosity. Green '+' circles = Low risk of Bias; yellow '?' circles = uncertainties about the risk of bias; red '−' circles = high risk of bias. The black rectangle represents the mean difference; The green dot and bar represent the overall summary estimate of the treatment effect, especially, the bar represents the confidence interval for the summary estimate. Reference: Bai 2008 [64]; Huang 2005 [68]; Yuan 2013 [61]; Zhang 2009 [59]; Lin 2017 [66]; Yin 2017 [67]; Guo 2007 [60]; Yang 2019 [62]; Jia 2017 [63]; Gu 2020 [65].

### 3.4.2. High Shear Rate

When compared with controls, HSR was significantly lower in the CDDP group (7 studies, MD −0.86, 95% CI −1.08 to −0.63) (Figure 5B) [59–61,64,66–68]. In subgroup analysis, four studies showed HSR was significantly lower in the CDDP + WM treatment group than in the WM control group (MD −1.03, 95% CI −1.16 to −0.90) [59,61,64,68]. In the two studies using CDDP vs. WM, HSR was significantly lower in the treatment group than in the control group (MD −0.84, 95% CI −1.09 to −0.59) [66,67]. In the one study comparing CDDP + WM vs. Placebo CDDP + WM, HSR was significantly lower in the treatment group than in the control group (MD −0.20, 95% CI −0.54 to 0.14) [60].

### 3.4.3. Plasma Viscosity

When the results of 10 studies measuring plasma viscosity (PV) were compared, the PV was lower in the treatment group than in the control group (MD −0.37, 95% CI −0.54 to −0.19) (Figure 5C) [59–68]. In subgroup analysis, six studies showed PV was significantly lower in the CDDP + WM treatment group than in the WM control group (MD −0.39, 95% CI −0.69 to −0.10) [59,61–64,68]. In the three studies using CDDP vs. WM, PV was significantly lower in the treatment group than in the control group (MD −0.34, 95% CI −0.61 to −0.08) [65–67]. In the one study using CDDP + WM vs. Placebo CDDP + WM, PV was significantly lower in the treatment group than in the control group (MD −0.22, 95% CI −0.57 to 0.13) [60].

### 3.5. Homocysteine

A meta-analysis of the eight studies measuring homocysteine showed lower homocysteine levels in the treatment group than in the control group (MD −8.32, 95% CI −9.05 to −7.58) (Figure 6) [51–58]. In subgroup analysis, homocysteine was significantly lower in the CDDP + WM treatment group than in the WM control group (n = 6, MD −8.23, 95% CI −9.08 to −7.38) [53–58]. Similarly, homocysteine was significantly lower in the CDDP treatment group than in the WM control group (n = 2, MD −8.55, 95% CI −9.99 to −7.12) [51,52]. No studies in the CDDP + WM vs. Placebo CDDP + WM subgroup reported homocysteine levels.
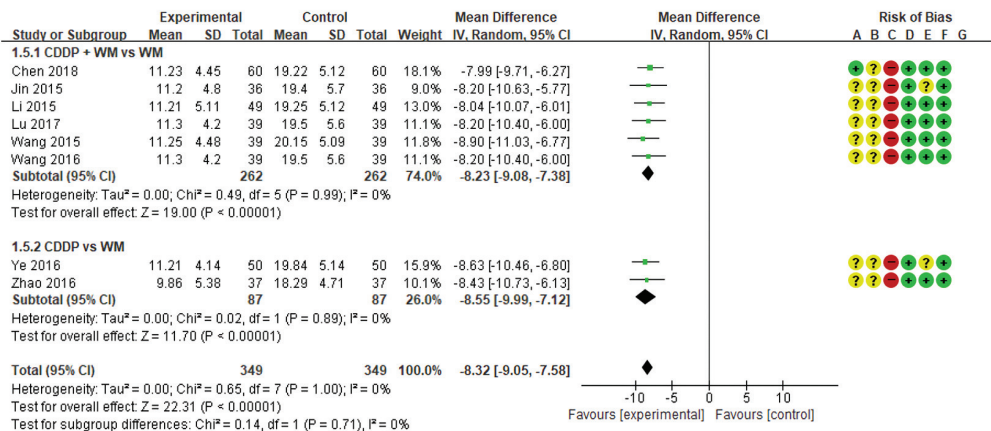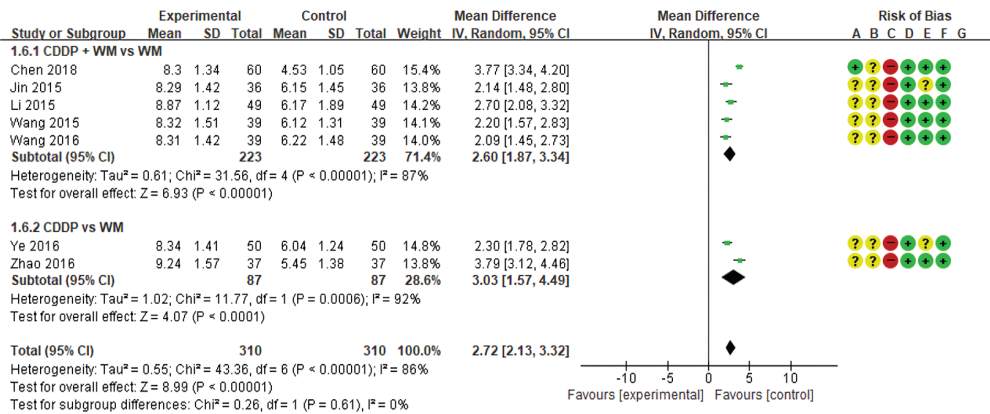


**Figure 6.** Meta-analysis of homocysteine between treatment and control groups. Green '+' circles = Low risk of Bias; yellow '?' circles = uncertainties about the risk of bias; red '−' circles = high risk of bias. The black rectangle represents the mean difference; The green dot and bar represent the overall summary estimate of the treatment effect, especially, the bar represents the confidence interval for the summary estimate. Reference: Chen 2018 [53]; Jin 2015 [54]; Li 2015 [56]; Lu 2017 [55]; Wang 2015 [58]; Wang 2016 [57]; Ye 2016 [51]; Zhao 2016 [52].

### 3.6. Plasma Adiponectin Levels

Eight studies measured plasma adiponectin levels. Among them, the study by Lu was excluded from the meta-analysis because the results suggested that there was an error in the notation of research data values [55]. When the analysis was conducted on the remaining seven studies, plasma adiponectin levels were higher in the treatment group than in the control group (MD 2.72, 95% CI 2.13 to 3.32) (Figure 7) [51–54,56–58]. In subgroup analysis, plasma adiponectin levels were significantly higher in the CDDP + WM treatment group than in the WM control group (n = 5, MD 2.60, 95% CI 1.87 to 3.34) [53,54,56–58]. Similarly, plasma adiponectin levels were significantly higher in the CDDP group than in the WM control group (n = 2, MD 3.03, 95% CI 1.57 to 4.49) [51,52]. No studies in the CDDP + WM vs. Placebo CDDP + WM subgroup reported plasma adiponectin levels.



**Figure 7.** Meta-analysis of plasma adiponectin levels between treatment and control groups. Green '+' circles = Low risk of Bias; yellow '?' circles = uncertainties about the risk of bias; red '−' circles = high risk of bias. The black rectangle represents the mean difference; The green dot and bar represent the overall summary estimate of the treatment effect, especially, the bar represents the confidence interval for the summary estimate. Reference: Chen 2018 [53]; Jin 2015 [54]; Li 2015 [56]; Wang 2015 [58]; Wang 2016 [57]; Ye 2016 [51]; Zhao 2016 [52].

### 3.7. Safety

There were 6/18 studies that measured adverse events (Supplementary S2) [53,59, 62,64,67,68]. One study reported no adverse events in both the treatment group and the control group [64]. In the remaining six studies, headache (n = 1), nausea (n = 1), dizziness (n = 1), and diarrhea (n = 1) were reported as adverse events in the treatment group. Overall, there were fewer adverse events in the treatment group than in the control group.

## 4. Discussion

### 4.1. Summary of Findings

In this systematic review and meta-analysis, the effectiveness of CDDP on the blood viscosity of patients with type 2 diabetes was searched in six databases. A total of 18 studies including 1532 DM patients were selected and investigated. The main findings of this review were that, compared to the WM control group, CDDP or CDDP + WM treatment in patients with T2DM resulted in significantly lower LSR, HSR, PV, and hemorheologic factors, such as homocysteine and adiponectin.

### 4.2. Importance of Blood Viscosity Control in DM Management

Diabetes mellitus is a type of metabolic disease in which the secretion or normal function of insulin is insufficient, and is characterized by an increase in the concentration of glucose in the blood, called hyperglycemia [69]. Chronic hyperglycemia damages

blood vessels and disrupts the formation of micro-vessels. For this reason, kidney or cardiovascular problems are common diabetic complications [70]. Diabetes is a chronic disease that is difficult to prevent, making management a social burden [5,71]. Therefore, the prevention and early management of diabetes is important [69]. Recently, studies have shown that elevated blood viscosity is frequently found in patients with diabetes, and they are among the causes of diabetic complications. Therefore, managing blood viscosity is helpful in preventing the progression or worsening of diabetes [19,72,73].

*4.3. Blood Viscosity, a Concept Similar to Blood Stasis, Is a Main Pathophysiology of East Asia Traditional Medicine*

In East Asian medicine, various attempts have been made to improve the condition of the blood. High blood viscosity, called "blood stasis" in traditional East Asian medicine, was thought to be one of the leading causes of chest pain and cerebral infarction and is listed in the International Classification of Diseases (ICD) code as "blood stasis pattern" or "blood stagnation pattern" [74,75]. This blood stasis pattern is derived from the concept of high blood viscosity and is believed to share similarities with the causes of metabolic and chronic pain diseases [76,77]. In particular, various drugs have been developed to improve blood conditions, including blood viscosity. CDDP, the target of this study, is a drug for reducing cardiovascular diseases, including hyperlipidemia and hypertension, and improves factors that affect blood viscosity [23,24,26]. In fact, CDDP is widely used to treat diabetic complications, diabetic retinopathy, hyperlipidemia, and coronary artery pathway conditions [26]. The main mechanisms of CDDP are microcirculatory recovery, anti-oxidative, anti-inflammatory, and inhibition of platelet adhesion and aggregation [24,78]. CDDP has completed Phase II clinical trials in the United States of America and China. Blood viscosity management is important; however, there is a lack of conventional medications with sufficient clinical evidence to support their effectiveness in treating blood viscosity [79]. Therefore, several alternative therapeutic options, such as exercise, diet, or yoga, are recommended to attempt to control blood viscosity [80–82].

*4.4. Predicted Mechanism Affecting Blood Viscosity-Related Factors in CDDP*

Various interventions have been utilized to regulate blood viscosity for disease prevention and treatment. Several reports have shown that natural products lower blood viscosity, and Galduróz et al. confirmed a decrease in blood viscosity after using Ginkgo biloba for 180 days [83]. In addition, animal experiments have demonstrated that *Salvia miltiorrhiza* extract lowers blood viscosity [84,85]. CDDP consists of 9 mg *S. miltiorrhiza*, along with 1.76 mg Panax notoginseng and Borneolum. Further, its major active constituents are tanshinol, protocatechuic aldehyde, salvianolic acid B, and notoginsenoside [86]. A previous study demonstrated a dose-dependent inhibition of Adenosine diphosphate(ADP)-induced platelet aggregation in vivo by salvianolic acid, a component of *S. miltiorrhiza* in CDDP. In addition, tanshinol shows similar results as aspirin in inhibiting Cox-2 and exerts its effect through the down-regulation of thromboxane B2 [87]. Unlike Aspirin, tanshinol has a protective effect against ulcer formation, which is advantageous over conventional aspirin therapy. As such, CDDP is expected to improve blood viscosity by reducing oxidative stress, increasing microcirculation, protecting blood vessels, and anti-inflammatory and anti-apoptotic effects [23,78]. Research on natural-based candidate substances for the treatment of diabetes complications is also conducted in silico, so future in-silico-based studies on the constituents of CDDP may aid in understanding their mechanisms [88]. In particular, as confirmed in this study, CDDP reduces the levels of homocysteine and plasma adiponectin. This may result from a multi-component, multi-target effect on the metabolism of these blood proteins, which is presumed to reduce blood viscosity and increase microcirculation. The summary of the mechanism of action of CDDP is depicted in Figure 8.
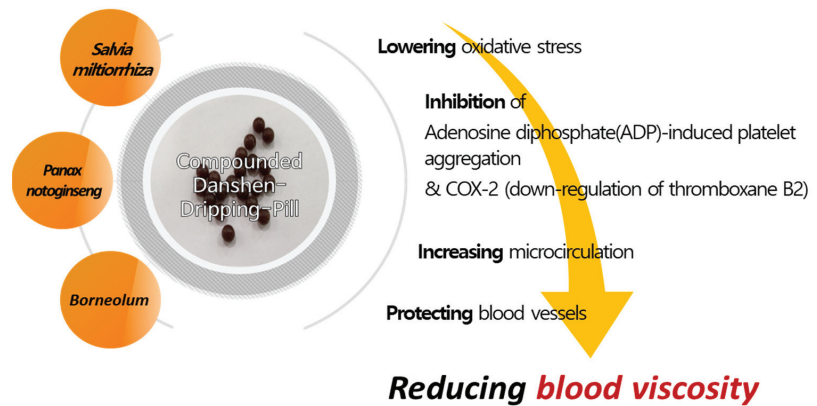
**Figure 8.** Possible mechanistic pathway of CDDP for reducing blood viscosity.

*4.5. Strengths*

This study had several limitations. Firstly, this study set blood viscosity as the primary outcome and did not verify the difference between hematocrit and fibrinogen, which are also important factors influencing blood viscosity. Because there are numerous factors that affect blood viscosity, it is necessary to conduct a meta-analysis on all factors affecting blood viscosity in the future. Moreover, blood viscosity itself has limitations; the normal range is still quite wide according to the current test method and the value is affected by various factors. Secondly, several included studies had various methodological flaws; therefore, there is a need for high-quality, well-designed studies in the future. Additionally, the information presented in some articles was inaccurate. For example, the study by Lu presented the SD of homocysteine as 11.25, which was higher than the average value of 9 [55]; it was hypothesized that the publication incorrectly indicated 1.25 as 11.25. However, we could not directly modify the RCT values of Lu while conducting the meta-analysis, so this study was excluded [55]. Thirdly, as this study targeted only type 2 diabetic patients, additional research is needed to determine if CDDP improves blood viscosity in diabetic patients with genetic predispositions, such as type 1 diabetes. Furthermore, we searched several core databases, including Pubmed, EMBASE, and CENTRAL, but we did not review all databases such as Web of Science. Therefore, it is possible that studies not included in our search may exist.

*4.6. Limitations*

There are also several strengths of our study. Although CDDP is widely used for the treatment of underlying diseases that affect blood viscosity, there has been no comprehensive review of the effectiveness of CDDP on blood viscosity. Therefore, this is the first study to identify studies in which blood viscosity was measured and assess the effectiveness and safety of CDDP in patients with diabetes by measuring blood viscosity. In addition, homocysteine and adiponectin, which are known to be related to blood viscosity, were evaluated as secondary outcomes, supporting the research results. In the case of diabetic patients included in the sample, it was observed that blood viscosity was higher in both systolic and diastolic periods than the normal reference value, and blood viscosity decreased after taking CDDP. Moreover, the homocysteine level was higher than the average value of 5–15 mmol/L, and the homocysteine level was significantly lower in the CDDP treatment group. These results are useful for supporting physicians in making clinical decisions.

*4.7. Implications for Clinical Practice and Future Research*

Homocysteine and plasma adiponectin levels, as well as values related to blood viscosity, are all affected by sex differences; however, these issues were not considered in this study. In future clinical studies that use CDDP as an intervention and blood viscosity

as the outcome, we recommend conducting a pilot study with healthy men aged 20–30 and excluding women whose blood test values may be affected by hormones produced during the menstrual cycle. More research is required to derive the normal range values for blood viscosity, especially classifying groups according to variables that may affect blood viscosity, including race, sex, age and obesity [89]. In addition, since most studies evaluated surrogate outcomes, it is necessary to plan RCTs that apply long-term follow-up clinical endpoints, such as diabetic complications or death. In terms of clinical practice, we believe that blood viscosity will become an increasingly important area in the future; blood viscosity values could be used to predict the occurrence risk of cardiovascular and cerebrovascular diseases. Our study suggests the possibility of CDDP as a candidate drug for blood viscosity management. Early prevention and management of complications in patients with diabetes is very important; therefore, prevention of vascular complications by concurrently administering CDDP as early as possible in patients with high blood viscosity may help to limit further complications from diabetes.

## 5. Conclusions

Our meta-analysis revealed the treatment with CDDP decreased blood viscosity in diabetic patients and reduced homocysteine and plasma adiponectin levels, which are factors that can affect blood viscosity, without severe adverse events. Therefore, CDDP combined treatment could be an effective and safe therapeutic method for controlling blood viscosity in patients with type 2 diabetes. However, as the methodological quality was relatively low, further well-designed, long-term follow-up clinical trials are required to improve confidence in this conclusion.

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MDPI

*Case Report*

# Hypoglycemic Effect of an Herbal Decoction (Modified Gangsimtang) in a Patient with Severe Type 2 Diabetes Mellitus Refusing Oral Anti-Diabetic Medication: A Case Report

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**Abstract:** There is growing interest in alternative therapies for type 2 diabetes mellitus (T2DM) because some patients refuse to receive conventional therapies. In East Asia, herbal medicines are often used to treat T2DM, and modified Gangsimtang (mGST) is prescribed to treat a condition called wasting thirst (消渴), which resembles T2DM. This study reported the treatment of hyperglycemia using herbal medicines without oral hypoglycemic agents or insulin therapy. *Case presentation*: A 36-year-old man with obesity was diagnosed with T2DM four years prior to hospitalization and experienced blood glucose level reduction from 22.2–27.8 mmol/L (400–500 mg/dL) to 5.6–11.1 mmol/L (100–200 mg/dL) by using herbal medicines. He visited D Korean Medicine Hospital with chronic polydipsia and general weakness as chief complaints. He was diagnosed with T2DM on the basis of a hemoglobin A1c level of 11.7% and 2 h postprandial blood glucose level of >25.0 mmol/L (450 mg/dL). Moreover, he was diagnosed with a "dual deficiency of qi and yin" (氣陰兩虛) because of ordinary symptoms (素證). During his 30-day inpatient treatment, the patient received mGST 120 mL thrice daily; as a result, his postprandial blood glucose level decreased from 25.3 mmol/L (455 mg/dL) to 8.6 mmol/L (154 mg/dL), polydipsia decreased (visual analog scale score decreased from six to one), and triglyceride levels decreased from 11.7 mmol/L (1031 mg/dL) to 2.0 mmol/L (174 mg/dL). Plasma glucose levels remained stable for 6 months after the treatment, and no adverse events were observed over 200 days. We administered an herbal decoction to decrease plasma glucose levels without using oral hypoglycemic agents or insulin. *Conclusions*: Herbal decoctions such as mGST can reduce hyperglycemia in patients with T2DM who refuse conventional therapy.

**Keywords:** diabetes mellitus; hyperglycemia; east Asian traditional medicine; integrative medicine; herbal medicine; case report

## 1. Introduction

Type 2 diabetes mellitus (T2DM) is characterized by hyperglycemia due to insulin resistance, comprises 90–95% of all diabetes cases [1], and manifests clinically as a metabolic syndrome [2]. Globally, the number of adults with T2DM has tripled in the last 20 years from 151 million in 2000 [3] to 537 million in 2021 [4].

Oral hypoglycemic agents are commonly administered for T2DM. Metformin, which is the first-line drug of choice, is known to cause side effects such as lactic acidosis [5] and vitamin B12 deficiency with long-term use [6]. Increasing the dose or administering combination therapy is common when monotherapy fails to achieve the desired effects [7]. Patients sometimes refuse to receive oral hypoglycemic agents or insulin owing to the inconvenience of receiving multiple medications and their various side effects [8]. Therefore, interest in alternative treatments, such as herbal medicines, is increasing [9].

In East Asia, the administration of herbal medications for treating T2DM is widespread and has a long history. Modern diabetes resembles the east Asian traditional medicine pathology of wasting thirst (WT, 消渴) [10]. The term WT was first used in *Huangdi's Internal Classic* (黃帝內經; fourth to second century BC), which is the oldest medical text in East Asia that discusses human anatomy, physiology, pathology, diagnosis, treatment, and prevention. The *Synopsis of Prescriptions of the Golden Chamber* (金匱要略; third century) first defined WT as a disease characterized by polydipsia, polyuria, and polyphagia, which are similar to the symptoms of the hyperglycemic hyperosmolar state of T2DM [11], and devoted a separate chapter to the treatment of WT. A combination of Western and east Asian traditional medicine has been widely used in clinical practice to improve blood glucose levels and relieve symptoms in patients with diabetes, with substantial clinical evidence [12–16].

The east Asian traditional medicine treatment is based on syndrome differentiation (辨證) according to the individual complaints of patients. Stomach heat syndrome (胃火熾盛證) and dual deficiency of qi and yin (DQY, 氣陰兩虛) are the most common conditions associated with T2DM [17]. DQY is characterized by dry mouth, dry throat, tired spirit, thirst, poor appetite, spontaneous perspiration, thin body, reddish tongue with less fur, and weak pulse. It is diagnosed when a patient shows three or more of the aforementioned symptoms [17]. Gangsimtang (GST) is an herbal decoction that tonifies DQY and is a prescription recorded in the *Treasured Mirror of Eastern Medicine* (東醫寶鑑) (1613) [18], which was written during the Joseon Dynasty and states the following: "Treats the desire to drink stemming from agitation (煩渴), daily consumption of qi-blood (氣血) due to a heart fire (心火) that flames upward, and impaired kidney water (腎水)". Trichosanthis Radix (TR) [19], Liriope Platyphylla [20], and Ginseng Radix [21], which are the constituent herbs of GST, have been used to manage blood sugar and reduce related symptoms in patients with diabetes.

Although studies and clinical trials on the use of herbal medicine for treating diabetes have been conducted, research is lacking on the therapeutic effects of herbal medicine without the use of oral hypoglycemic agents or injections in patients with severe hyperglycemia (postprandial plasma glucose level > 25.0 mmol/L). Furthermore, no clinical study has reported the hypoglycemic effect of GST on patients with T2DM. In the current study, we presented a case of a patient who refused conventional medications and was administered GST with dietary restrictions. The treatment resolved hyperglycemic symptoms, reduced blood glucose levels without oral hypoglycemic agents or insulin injections, and maintained optimal plasma glucose levels during a long-term telephone follow-up period.

## 2. Case Presentation

### 2.1. Patient History

We present a case of T2DM in a 36-year-old man with a height of 176 cm, weight of 84 kg, and body mass index (BMI) of 27.1 kg/m$^2$ who worked in an office and had low levels of physical activity and irregular eating habits. In 2018, the patient was diagnosed with T2DM at K Korean Medicine Hospital on the basis of a blood glucose level of 22.2–27.8 mmol/L (400–500 mg/dL) and symptoms of general weakness, polydipsia, and polyuria. He was hospitalized for 3 weeks and treated with herbal medicines and acupuncture. Owing to the fear of having to receive oral anti-diabetic medication for the rest of his life, he only received herbal medicine and acupuncture. After inpatient treatment, his blood sugar level was successfully maintained within the range of 5.6–11.1 mmol/L

(100–200 mg/dL), leading to his discharge from the hospital with alleviated symptoms. He did not modify his lifestyle or receive medications or insulin treatment for hyperglycemia in the subsequent four years.

In January 2022, the patient was diagnosed with coronavirus disease 2019 and received only symptomatic treatment. In the same year, he was diagnosed with gastroesophageal reflux disease and colon polyps, underwent polypectomy, and received oral medications. When he visited D Korean Medicine Hospital in April 2022, he did not complain of any respiratory or digestive symptoms related to his past medical history. In March 2022, he developed cervical pain caused by prolonged working hours in front of a computer. In April 2022 (10 days before Day 1 of hospitalization), the cervical pain worsened; thus, he underwent acupuncture, cupping, and moxibustion treatment at D Korean Medicine Hospital. During his hospitalization period, the patient received traditional Korean medicine twice daily (acupuncture, moxibustion, and cupping) and extracorporeal shock wave treatment.

*2.2. Diagnostic Approach in Conventional and East Asian Traditional Medicine*

In April 2022 (Day 1), the patient was admitted to D Korean Medicine Hospital with polydipsia and general weakness as chief complaints. On Day 1, diabetes was diagnosed on the basis of a blood test that revealed a plasma glucose level of 20.9 mmol/L (377 mg/dL) and a hemoglobin A1c (HbA1c) level of 11.7%, as measured using the certified method of the National Glycohemoglobin Standardization Program (NGSP). In 2013, the prevalence of type 1 diabetes mellitus (T1DM) in Korea was 46.66 in 100,000 individuals, and the incidence of T1DM in East Asia is among the lowest worldwide [22,23]. The patient had no history of autoimmune diseases, including Hashimoto's thyroiditis, Graves' disease, celiac disease, or T1DM. In addition, there was no catabolic presentation, such as unintentional weight loss or ketonuria, and no evidence or history of diabetic ketoacidosis (DKA). Therefore, T1DM was excluded in this patient. Drug-induced diabetes was ruled out because the patient had not received any medications, including glucocorticoids, within the past 1 month. Diseases of the exocrine pancreas were excluded because no other digestive diseases, such as cystic fibrosis or pancreatitis, were diagnosed at the time of polypectomy for colon polyp at the gastroenterology department in January 2022. Moreover, given that there was no family history of diabetes, monogenic diabetes syndrome was excluded.

The patient was diagnosed with T2DM at a different hospital in 2018 owing to insulin resistance because he had obesity with a BMI score of 27.1 kg/m$^2$, a triglyceride level of 11.7 mmol/L (1031 mg/dL), and hypertension ($\geq$130/85 mmHg) on Day 1, which implied that the patient had a metabolic syndrome according to the criteria of the National Cholesterol Education Program Adult Treatment Panel III. He was hospitalized for 30 days from April 2022 (Day 1) to May 2022 (Day 30) and was treated using an herbal decoction and dietary restrictions.

The patient complained of frequent fatigue, dry mouth, dry throat, and thirst. Additionally, he complained of spontaneous perspiration. On examination, he had a reddish tongue with less fur. Six of the eight symptoms, including reddish tongue and weak pulse, were present, thus leading to the diagnosis of DQY (Table 1).

**Table 1.** Symptoms and diagnostic criteria of the dual deficiency of qi and yin (氣陰兩虛). A dual deficiency of qi and yin is diagnosed when a patient has three or more symptoms with a reddish tongue and weak pulse.

| Symptoms | Symptoms in This Case | Symptoms | Symptoms in This Case |
|---|---|---|---|
| Dry mouth | (+) | Poor appetite | (−) |
| Dry throat | (+) | Spontaneous perspiration | (+) |
| Tired spirit | (+) | Thin body | (−) |
| Thirsty | (+) | Less tongue fur | (+) |

### 2.3. Dietary Restriction

During hospitalization, the patient received approximately 1200 calories daily. The patient did not usually have dietary restrictions and consumed a variety of high-calorie snacks. He was allowed to eat one vegetable per day, such as a cucumber or a tomato, to relieve hunger because of dietary restrictions, but no other snacks were allowed.

### 2.4. Herbal Decoction Therapy

Syndrome differentiation (pattern identification) according to east Asian traditional medicine theory led to a diagnosis of DQY (氣陰兩虛). The patient complained of insomnia and elevated blood glucose levels. Therefore, GST was prescribed for DQY and insomnia [18]. GST contains TR (天花粉), recognized as a sovereign medicine (君藥) and a key ingredient in herbal decoction [24] and known for its hypoglycemic effects [19,25]. Liriope Platyphylla (麥門冬) in the mixture has tonifying yin action, while Ginseng Radix (人蔘) has tonifying yin and qi actions. These herbs were administered as part of the modified GST (mGST), which was increased by 50% in the prescription from 8 to 12 g daily, brewed to 360 mL daily, and administered thrice daily starting from Day 4 (120 mL for each administration).

After administering mGST, the 2 h postprandial plasma glucose test showed a slight decrease from 25.0 mmol/L (450 mg/dL) on Day 1 to 17.8 mmol/L (320 mg/dL) on Day 8; thus, TR was increased by 50% from 16 to 24 g/day to further reduce the blood glucose level. To reduce polydipsia, Peurariae Radix (葛根) [14], which acts as an engender fluid (生津) and is commonly used for diabetes mellitus treatment, was prescribed in addition to 8 g of mGST daily and administered in the same dosage from Day 9 (Day 6 of mGST) (Table S1). The patient adhered to the intervention schedule and tolerated the herbal decoction.

### 3. Results

#### 3.1. Changes in the Objective Test Results and Subjective Symptoms (Figure 1)

As a result of treatment, the 2 h postprandial plasma glucose levels decreased from 25.3 mmol/L (455 mg/dL) on Day 1 to 8.5 mmol/L (154 mg/dL) on Day 29, and the fasting plasma glucose levels decreased from 15.0 mmol/L (270 mg/dL) on Day 2 to 7.8 mmol/L (141 mg/dL) on Day 30. (Table 2) We performed five blood tests at a 7-day interval for 30 days, with blood drawn in a fasting state after a 10 h fast. HbA1c was measured using an NGSP-certified method, which revealed that HbA1c decreased from 11.7% on Day 1 to 10.7% on Day 21; this result represented a 1.0% decrease over 20 days. The triglyceride level was 11.7 mmol/L (1035 mg/dL) on Day 1 but stabilized between 1.7 and 2.4 mmol/L (149–210 mg/dL) from the second blood test on Day 8 to the fifth blood test on Day 28. Urinalysis showed glucose 4+ and ketones 2+ on Day 1, which improved to glucose (−) and ketones (+/−) on Day 28 (Table 3).

**Table 2.** Change in Plasma Glucose.

| (mmol/L) | 6 a.m. (Fasting Plasma Glucose) | 2 p.m. (2 h Postprandial Plasma Glucose) | 4 p.m. (4 h Postprandial Plasma Glucose) | 9 p.m. (3 h Postprandial Plasma Glucose) |
|---|---|---|---|---|
| Day 1 | | 25.3 *** | 21.0 ** | 25.4 *** |
| Day 2 | 15.0 * | 25.8 *** | 20.6 ** | 19.9 ** |
| Day 3 | 14.4 * | 19.5 ** | 15.5 * | 19.0 ** |
| Day 4 | 15.8 * | 19.3 ** | 13.8 * | 16.6 * |
| Day 5 | 13.9 * | 16.5 * | 15.9 * | 16.1 * |
| Day 6 | 12.9 * | 17.0 ** | 16.1 * | 14.4 * |
| Day 7 | 13.7 * | 17.8 ** | 15.7 * | 14.4 * |
| Day 8 | 10.1 | 17.7 ** | 13.7 * | 11.7 * |
| Day 9 | 11.1 * | 14.0 * | 12.2 * | 14.3 * |
| Day 10 | 10.4 | 15.4 * | 15.4 * | 13.3 * |

**Table 2.** *Cont.*

| (mmol/L) | 6 a.m.<br>(Fasting Plasma<br>Glucose) | 2 p.m.<br>(2 h Postprandial<br>Plasma Glucose) | 4 p.m.<br>(4 h Postprandial<br>Plasma Glucose) | 9 p.m.<br>(3 h Postprandial<br>Plasma Glucose) |
|---|---|---|---|---|
| Day 11 | 9.2 | 13.0 * | 11.1 * | 15.9 * |
| Day 12 | 11.6 * | 12.5 * | 12.4 * | 16.4 * |
| Day 13 | 12.3 * | 13.1 * | 11.1 * | 12.3 * |
| Day 14 | 9.7 | 10.0 | 9.4 | 15.1 * |
| Day 15 | 10.0 | 11.3 * | 13.9 * | 11.4 * |
| Day 16 | 9.0 | 11.4 * | 8.3 | 11.5 * |
| Day 17 | 9.5 | 10.8 | 11.2 * | 8.8 |
| Day 18 | 8.4 | 10.0 | 10.6 | 11.7 * |
| Day 19 | 9.2 | 10.2 | 12.1 * | 11.8 * |
| Day 20 | 7.4 | 8.3 | 7.8 | 11.4 * |
| Day 21 | 8.1 | 10.5 | 9.0 | 11.0 |
| Day 22 | 8.8 | 11.2 * | 9.2 | 9.9 |
| Day 23 | 9.1 | 8.9 | 7.9 | 9.6 |
| Day 24 | 8.4 | 8.0 | 8.0 | 5.8 |
| Day 25 | 6.8 | 10.2 | 9.9 | 8.9 |
| Day 26 | 8.5 | 12.7 * | 9.4 | 8.1 |
| Day 27 | 7.8 | 8.9 | 7.8 | 9.4 |
| Day 28 | 8.0 | 8.3 | 7.5 | 11.0 |
| Day 29 | 8.3 | 8.5 | 6.8 | 9.0 |
| Day 30 | 7.8 | | | |

*** >22.2 mmol/L [400 mg/dL], ** 16.7–22.1 mmol/L [300–399 mg/dL], and * 11.1–22.0 mmol/L [200–299 mg/dL].
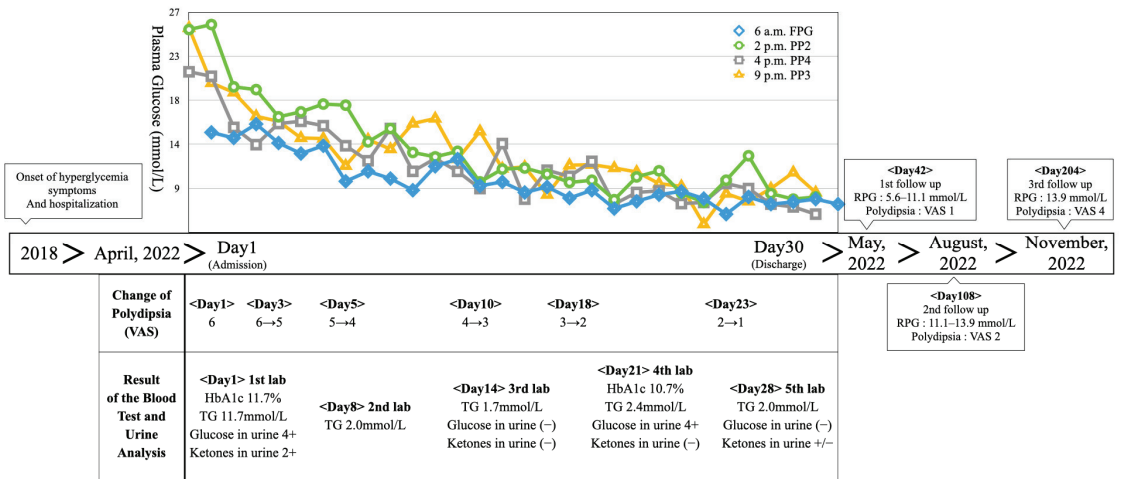


**Figure 1.** Timeline of the case. Abbreviations: FPG—fasting plasma glucose; PP2—2 h postprandial plasma glucose; PP3—3 h postprandial plasma glucose; PP4—4 h postprandial plasma glucose; VAS—visual analog scale; TG—triglyceride; HbA1c—hemoglobin A1c; RPG—random plasma glucose.

**Table 3.** Results of the blood test and urine analysis. Blood samples were taken after 10 h of fasting. HDL: high-density lipoprotein.

|  | Reference Value | Day 1 | Day 8 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|---|
| HbA1c in % | 4.0–5.6 | 11.7 * |  |  | 10.7 * |  |
| Plasma glucose in mmol/L (mg/dL) | 3.9–6.1 (70–110) | 20.9 * (377) * | 18.7 * (336) * | 10.8 * (194) * | 9.3 * (167) * | 7.3 * (131) * |
| Cholesterol total in mmol/L (mg/dL) | 3.5–5.7 136–220 | 6.7 * (260) * | 7.4 * (286) * | 6.4 * (247) * | 6.4 * (245) * | 6.1 * (234) * |
| Triglyceride in mmol/L (mg/dL) | 0.1–1.7 (10–149) | 11.7 * (1031) * | 2.0 * (176) * | 1.7 (149) | 2.4 * (210) * | 2.0 * (174) * |
| Glucose in urine | - | 4+ * | Non-excution | - | 4+ * | - |
| Ketones in urine | - | 2+ * | Non-excution | - | - | +/− * |

\* out-of-reference value.

Polydipsia was assessed using a visual analog scale (VAS), which showed a score of six on Day 1. The patient complained of continuous thirst even after occasionally drinking water. The patient drank approximately 4 L of water daily, excluding meals. During his hospitalization, his self-reported thirst decreased along with his plasma glucose level; this led to a decrease in the VAS score to four on Day 5. Furthermore, his drinking volume decreased to 2 L. The patient's symptoms continued to decrease, with a VAS score of one on Day 23, which was described as barely noticeable. The VAS score remained at one until discharge on Day 30.

From Days 1 to 3, before receiving mGST, the patient slept for an average of 4.7 h per night and complained of insomnia with lethargy and frequent awakenings. However, from Days 27 to 29, he slept for an average of 6 h per night with no interruptions (Figure 2).
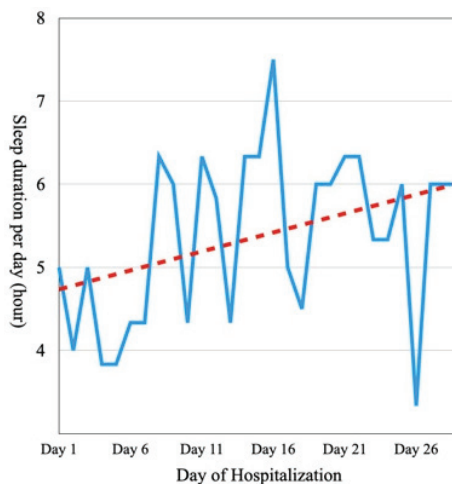


**Figure 2.** Sleep duration per day during hospitalization. The dotted line is the trend line obtained by linear regression of the sleep duration per day.

After discharge on Day 30, the patient did not receive herbal or acupuncture treatment. The patient visited D Korean Medicine Hospital on Day 42, which was 12 days after discharge. A self-measured plasma glucose test at home showed a distribution of 5.6–11.1 mmol/L (100–200 mg/dL). Associated symptoms, such as polydipsia and polyuria, remained unremarkable. On Day 108, the patient was followed up with via telephone. It

was reported that he could not control his diet because of his corporate lifestyle. However, the amount of food he ate was similar to that during hospitalization (approximately half of his normal diet), and his plasma glucose level was maintained at 11.1–13.9 mmol/L (200–250 mg/dL) according to a random plasma glucose test. The polydipsia symptoms had increased slightly since the time of discharge. However, he reported a VAS score of two, which did not significantly interfere with his daily activities. The urinary frequency was maintained at 10 times daily, including 1–2 episodes of nocturia. No other side effects, such as hypoglycemia or weight gain, were observed. On Day 204, the patient was monitored via telephone. It was reported that he was consuming approximately half of his normal diet, and his plasma glucose level was randomly tested two to three times a week and was maintained at approximately 13.9 mmol/L (250 mg/dL). He reported an increase in polydipsia (VAS score of four); however, this did not significantly interfere with his daily life.

The gastrointestinal symptoms commonly associated with herbal medicines, such as heartburn, diarrhea, and nausea, were not reported until the Day 204 follow-up, spanning the entire treatment duration. Blood tests, which were conducted weekly during the hospitalization, included assessments of complete blood count, electrolyte levels, liver function (as indicated by aspartate transaminase, alanine transaminase, and γ-glutamyl transferase), and kidney function (evaluated through blood urea nitrogen and blood creatinine levels). All test results fell within the reference ranges and exhibited no abnormalities, indicating the absence of adverse effects, such as drug-induced liver injury or drug-induced kidney injury.

### 3.2. Patient Perspective

Table 4 shows the patient's perspective on controlling blood sugar and hyperglycemic symptoms with herbal decoctions only on the basis of the patient's willingness to forego conventional medication.

**Table 4.** Patient perspective.

| Date | Situation | Patient's Perspective |
|---|---|---|
| A day before Day 1 | In an outpatient visit for counseling to manage plasma glucose and reduce the symptoms of polydipsia and polyuria. | "I have heard that once you start taking conventional drugs, you have to take them for the rest of your life, so I want to avoid them as much as possible. I have also heard that they have a lot of side effects, and I have heard that even if I take them, my plasma glucose will not be well controlled, so I am reluctant to take them. I have heard that herbal medicines have fewer side effects, so I want to be treated with herbal medicines". |
| Day 2 | After a blood test on Day 1 | "I thought my levels (of blood glucose, triglycerides, etc.) would be bad, but they are worse than I thought. In 2018, my levels were this bad, but I was treated at a traditional Korean medicine clinic and got better. So I think I will get better with traditional Korean medicine this time". |
| Day 10 | Polydipsia symptoms decreased from a VAS of six (Day 1) to a VAS of three. | "My mouth feels half as dry as it did on my first day in the hospital. I used to go to the bathroom every 3 h, but now it is more like every 4–5 h. I eat less than I used to so I am hungry, but it is manageable. I think I can keep it under control, and I am relieved that my plasma glucose levels continue to improve". |

**Table 4.** *Cont.*

| Date | Situation | Patient's Perspective |
|---|---|---|
| Day 28 | After the discharge decision after the fifth blood test. | "My symptoms of dry mouth and frequent urination have almost disappeared, and I am sleeping better at night and waking up less often than I did at the beginning of my hospitalization. I am glad that my blood glucose levels have improved without conventional medication and that my symptoms have improved so much. I will continue to test my plasma glucose as you taught me after I leave the hospital. I am grateful that my overall condition has improved so much without any side effects during my hospitalization". |
| August 2022 (Day 108) | Follow-up over the telephone | "It is not as easy to manage my diet and lifestyle as it was when I was hospitalized because I am living a social life, but when I check my blood glucose often, my blood glucose level is lower than before I was hospitalized (around 11.1–13.9 mmol/L). My symptoms of dry mouth and frequent urination have remained improved, so it's much easier for me to go about my daily life". |
| November 2022 (Day 204) | Follow-up over the telephone | "I have been out of the hospital for about six months now, and my symptoms have not gotten much worse in my daily life. I try, but I am too busy and frustrated to keep up with my diet, but I am still in better condition than I was before I was hospitalized. My blood glucose seems to be a little high, but if it gets higher or my symptoms get worse, I will go back to the doctor for herbal medicine". |

## 4. Discussion

### 4.1. Summary of Findings

We presented a case of a patient who was prescribed oral medication after being diagnosed with T2DM but preferred to use herbal decoctions only. He was hospitalized in a Korean hospital for 30 days, during which herbal decoctions and dietary management reduced his blood sugar levels and alleviated his symptoms. To our knowledge, only one case report has been conducted [26] on glycemic lowering and symptomatic improvement using herbal medicine alone in patients with severe diabetes mellitus; symptoms of hyperglycemia, HbA1c $\geq$ 10%, and plasma glucose level $\geq$ 16.7 mmol/L (300 mg/dL); and requiring early insulin treatment [7]. In the future, herbal decoctions may be considered for patients with severe diabetes who do not respond to conventional treatments.

### 4.2. Suggested Mechanism of Hypoglycemic Effect

A previous animal study [27] reported that GST extracts significantly decreased plasma glucose levels in hyperglycemic rats compared with the control group. TR, which is the sovereign medicine (君藥) in GST that activates phosphatase on insulin receptors, is the most commonly administered herbal medication for diabetes in Taiwan [19]. Animal studies have identified proteins with hypoglycemic effects in TR [19] and have shown that TR improves renal function in a dose-dependent manner in streptozotocin-induced renal impairment [25]. TR is also used to treat and restore renal function impairment in patients with diabetes. Pueraria lobata is an herbal medication that has been used in East Asia for thousands of years for diabetes treatment and blood sugar level control of patients with T2DM [14,28]. Liriope Platyphylla regulates lipogenesis and lipid uptake in animal studies [29], stimulates insulin secretion from pancreatic beta cells, and reduces abdominal fat deposition [20]. Ginseng Radix reduces the levels of fasting plasma glucose, total

cholesterol, interleukin-6, and homeostatic model assessment of insulin resistance [21]. In addition, ginsenoside extracted from ginseng has been used as an adjuvant for patients with diabetes mellitus [30]. The various biologically active constituents of Astragali Radix can protect islet beta cells, reduce plasma glucose, and inhibit insulin resistance [15,31]. Although the diabetic effects of each herbal medication constituting GST are known, data on the diabetic effects of GST, which is a complex herbal formula, have been lacking in previous studies. In the current study, we demonstrated the effect of GST on decreasing blood glucose levels and reducing hyperglycemic symptoms.

Personalized east Asian traditional medicine is one important contributor to this hypoglycemic effect. The diagnosis is based on a comprehensive evaluation of ordinary symptoms (素證) such as food intake and digestion, sleep, and fatigue and symptoms such as tongue conditions and pulse characteristics. DQY causes dry mouth, dry throat, tired spirit, thirst, poor appetite, spontaneous perspiration, weight loss, reddish tongue, less fur, and weak pulse [17,32]. Our patient was initially diagnosed with yin deficiency and heat exuberance (陰虛熱盛) and was prescribed Galgeungeumryeontang (葛根黄芩黄連湯, Gegen Qinlian decoction). However, after observation, this medicine was deemed to be more appropriate for DQY; thus, the prescription was changed to mGST on Day 4 of hospitalization. TR was increased to manage hyperglycemia. The patient had symptoms of reddish tongue, weak pulse, fatigue, and spontaneous perspiration; was diagnosed with DQY; and was prescribed mGST, which is a tonifying yin and qi formula with TR as the sovereign medicine (君藥).

### 4.3. Strengths and Limitations

This study has several limitations. First, it was a single case report; therefore, the effect of mGST requires further study. Second, measurements of serum ketone levels, bicarbonate concentration, blood pH, autoantibody tests, or C-peptide were not conducted. Consequently, the exclusion diagnosis of the T1DM or ketoacidosis exclusion relied solely on clinical evaluation. Third, the hypoglycemic effect was interpreted as a synergistic effect of the herbal decoction and dietary control and was not due to the herbal decoction alone. According to a systematic review and meta-analysis of weight-loss interventions [33], the Mediterranean-style diet study group [34] had the highest HbA1c level reduction of 1.2% over 12 months. By contrast, other diets showed a reduction of ≤0.6%. Therefore, the glycemic lowering effect in this study, including a 1.0% reduction in HbA1c levels after 20 days of hospitalization, is thought to be caused by the synergistic effect of the herbal decoction and dietary control.

Acupuncture could have an effect on reducing blood glucose. However, according to the meta-analysis about acupuncture for T2DM [35], the major acupoints for treating T2DM include ST 36, LI 11, SP 6, BL 20, and LI 4. The patient in this case report had acupuncture therapy only for his cervicalgia. Thus, we used acupoint in his neck muscles such as the scalene, trapezius, and levator scapulae muscles, not acupoint for treating internal diseases like T2DM. Therefore, the hypoglycemic effect in this case was likely caused by the herbal medicine rather than the acupuncture treatment.

This case report has several strengths. First, the patient was under dietary control during hospitalization and was continuously monitored using blood glucose tests four times a day and weekly blood tests. Second, the prescription was personalized by a specialist in traditional Korean medicine with >10 years of clinical experience. Third, although previous studies on the blood sugar–lowering effects of herbs such as TR, Liriope Platyphylla, Ginseng Radix, and Peurariae Radix have been conducted, no study has reported on GST, except for some animal studies [27]. Therefore, the current study is the first case report on GST.

Finally, in a previous case study [26], herbal medicine alone was administered to manage severe diabetes with an HbA1c level of >10%. However, that study was conducted within 1 month from the initial diagnosis of T2DM, and it was impossible to determine the efficacy of a specific herbal prescription during the administration of multiple

herbal medications during the treatment period. In the current study, the patient was diagnosed with T2DM more than four years ago and probably had relatively high insulin resistance. Notably, unlike previous studies, this study used a single mGST prescription. In our knowledge, this is the first case report on GST that identified the detailed glycemic changes during hospitalization. In addition, this study explored the therapeutic window of mGST by confirming that no adverse events occurred for 200 days post-treatment. The self-measured plasma glucose tests after discharge showed that blood glucose was maintained to some extent after herbal medicine discontinuation. No dietary restrictions were imposed via telephone follow-up.

### 4.4. Implications for Clinical Practice and Further Study

Given that GST is a prescription recorded in the *Treasured Mirror of Eastern Medicine* (東醫寶鑑) (1613) [18], it is a commonly used prescription in Korea but not in China, Taiwan, or Japan. Therefore, few clinical reports have been conducted on GST for diabetes management, and future clinical studies are required. Case series or prospective observational studies are crucial to investigate the long-term effects of herbal medicine on weight control and diabetes remission, as reported in a previous study that reported diabetes remission via weight control [36]. Because the patient in this case was treated with a combination of dietary restriction, acupuncture treatment, and herbal medicine, it is difficult to reliably distinguish the effects of each intervention in this study. Therefore, further studies are needed to confirm the effects of each intervention and the synergetic effects of these interventions.

Because T2DM is a progressive disease, it is possible for patients to fail to respond to conventional treatment [7]. As this study indicated the effectiveness of herbal medicine in a patient who refused conventional therapy, it is recommended to confirm its effectiveness in patients who do not respond to conventional therapy in the future.

HbA1c tests should be performed at 12-week intervals [37]. Even though the tests were performed only 3 weeks apart in this case, a significant decrease was achieved. Future studies should include HbA1c measurements at intervals of 12 weeks or more to confirm continuous hypoglycemic effects.

DKA stands out as a critical complication of diabetes [38]. In this case, the patient underwent urine ketone measurement but lacked assessment of serum ketone levels, bicarbonate concentration, or pH, which resulted in an incomplete screening for DKA. However, given the patient's stable vital signs and the absence of clinical symptoms indicative of DKA, the likelihood of DKA is low. As with type 1 diabetes [39], it is worth exploring herbal medicine treatments for DKA in type 2 diabetes through further research.

### 4.5. Suggested Algorithm for Treating Patients Refusing Conventional Therapy

Even though, there are alternative treatments for diabetes mellitus that can be used for patients who refuse conventional therapy. However, in severe cases of diabetes, it is important to use conventional therapy, including insulin injections, to prevent critical complications such as DKA [38]. Given the substantial evidence supporting conventional therapy for patients with T2DM, it is crucial to prioritize the use of interventions such as psychological intervention [40] to encourage patients to undergo conventional therapy, thus preventing critical complications. In the case of patients who persistently refuse conventional therapy, hypoglycemic effects can be achieved through treatments such as herbal medicines with continuous monitoring of blood glucose and vital signs [41]. Above all, strict dietary management and other lifestyle changes are imperative and should be prioritized [35,42].

### 5. Conclusions

In this case report, a patient with severe T2DM, hyperglycemia, and hypertriglyceridemia with hyperglycemic symptoms, such as polydipsia and polyuria, who refused conventional medication was successfully treated with mGST based on TR to decrease

plasma glucose levels. After being discharged from the hospital, the patient's plasma glucose level remained stable via lifestyle management without herbal medicine for 6 months, thus indicating that the effect of the mGST treatment lasted for approximately 6 months; no side effects were observed. For patients with T2DM who refuse conventional treatment, mGST can be considered for glycemic control.

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*Review*

# A Review and Meta-Analysis of the Safety and Efficacy of Using Glucagon-like Peptide-1 Receptor Agonists

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**Abstract:** Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) have been used to reduce glucose levels in patients with type 2 diabetes mellitus since 2005. This meta-analysis discusses the mechanisms and potential benefits of several GLP-1 RAs. In particular, this meta-analysis focuses on the safety and associations with weight loss, glucose reduction, cardiovascular outcomes, heart failure, and renal outcomes of GLP-1 RAs to determine their benefits for patients with different conditions. In terms of glycemic control and weight loss, semaglutide was statistically superior to other GLP-1 RAs. In terms of cardiovascular outcomes, 14 mg of semaglutide taken orally once daily and 1.8 mg of liraglutide injected once daily reduced the incidence of cardiovascular death, whereas other GLP-1 RAs did not provide similar benefits. Moreover, semaglutide was associated with superior outcomes for heart failure and cardiovascular death in non-diabetic obesity patients, whereas liraglutide worsened heart failure outcomes in diabetic patients with a reduced ejection fraction. Additionally, semaglutide, dulaglutide, and liraglutide were beneficial in terms of composite renal outcomes: These GLP-1 RAs were significantly associated with less new or persistent macroalbuminuria, but not with improved eGFR deterioration or reduced requirement for renal replacement therapy. However, GLP-1 RAs may benefit patients with type 2 diabetes mellitus or obesity.

**Keywords:** GLP-1; diabetes; insulin; cardiovascular; renal

## 1. Introduction

Type 2 diabetes mellitus (T2DM) has been steadily increasing in prevalence globally. In response to this critical health concern, researchers have developed novel oral glucose-lowering agents, including glucagon-like peptide-1 receptor agonists (GLP-1 RAs) [1,2]. GLP-1 RAs can reduce glycated hemoglobin (Hb1Ac), improving glycemic control and reducing weight [3]. Additionally, GLP-1 RAs are associated with a low risk of hypoglycemic episodes [4]. Moreover, according to the 2023 guidelines of the American Diabetes Association, GLP-1 RAs are associated with numerous cardiovascular benefits in patients with T2DM with comorbidities of established atherosclerotic cardiovascular diseases (AS-CVDs) [5]. This review introduces the mechanisms, development history, and current clinical applications of GLP-1 RAs [6].

## 2. Physiology of Glucagon-like Peptide-1 (GLP-1)

GLP-1 is produced in enteroendocrine L-cells of the distal small bowel and colon. Concentrations of GLP-1 are low during fasting and high after meals [7]. GLP-1 is produced

in the intestines after food intake and activated by DPP-4 enzyme cleavage. Although GLP-1 is released rapidly, it has a short half-life of approximately 2 min [8]. After DPP-4 cleavage, GLP-1 is released biphasically. During the rapid first phase, GLP is released into the bloodstream within 15–30 min of nutrient ingestion. In the second, more gradual phase, a minor peak in bloodstream GLP-1 levels occurs between 90 and 120 min after nutrient ingestion. Multiple agents have been demonstrated to affect GLP-1 secretion, consisting of fat, gamma-aminobutyric acid, glycine, and somatostatin [9,10].

As GLP-1 binds to its receptors, adenylate cyclase is activated, elevating cyclic adenosine monophosphate (cAMP) levels. Elevated cAMP levels increase the concentrations of protein kinase A (PKA) and cAMP-regulated guanine nucleotide exchange factor 2 (cAMP-GEF2), causing a cascade of reactions that ultimately increases cytoplasmic $Ca^{+2}$, inducing exocytotic insulin release from insulin granules and mitochondrial adenosine triphosphate (ATP) synthesis [11,12].

In normal beta cells, insulin is influenced by blood sugar levels in a linear fashion. Insulin release occurs in two phases. During the first phase, which lasts approximately 10 min, hepatic glucose production is suppressed. During the second phase, which lasts approximately 2 h, insulin is released into the bloodstream. However, in patients with T2DM, the first phase does not occur or occurs with hepatic glucose suppression, delaying and impeding the onset of the second phase and allowing increased insulin levels to accumulate in the bloodstream. Excess insulin production to overcome insulin resistance causes healthy beta cells to be gradually replaced with amyloids [13,14]; once patients are clinically diagnosed with T2DM, they retain only approximately 50% of normal beta cell function [15]. A decline in beta cell function contributes to the failure of many biological processes that promote long-term glycemic control [16]. GLP-1 RAs counteract this decline by improving insulin resistance and glucose homeostasis [17].

GLP-1 is also associated with weight loss in clinical trials [18–21]. The mechanism through which GLP-1 induces weight loss involves several hypothalamic nuclei (the arcuate nucleus of the hypothalamus, the periventricular hypothalamus, and the lateral hypothalamic area) and hindbrain nuclei (the parabrachial nucleus and the medial nucleus tractus solitarius), in addition to the hippocampus (the ventral subregion) and the nuclei embedded within the mesolimbic reward circuitry (the ventral tegmental area and the nucleus accumbens) [22]. One study conducted two experiments demonstrating that the activation of GLP-1 in the subdiaphragmatic vagal afferents and the brain contributes to the intake-inhibitory effects of GLP-1 RAs [23]. Consequently, GLP-1 RAs suppress metabolism and appetite, leading to body weight loss.

## 3. General Effects and Developments

GLP-1 regulates blood sugar levels by promoting insulin production and inhibiting glucagon. Additionally, GLP-1 limits weight gain by suppressing gastric emptying and appetite [24,25]. The first GLP-RA to be available commercially was exenatide, approved in 2005 by the United States Food and Drug Administration (US FDA) [26]. The second GLP-1 RA to be commercially available was liraglutide, approved in 2009, which was designed to be similar to mammalian GLP-1 [27]. Liraglutide binds free fatty acids to plasma albumin and intestinal fluids; the resulting albumin-bound reservoir prolongs the medication's effects. Moreover, liraglutide has an elimination half-life of approximately 13 h, making it suitable for once-daily injection [28,29]. Subsequent advances in GLP-1 RAs extended this half-life, utilizing large protein-bound compounds such as dulaglutide or efpeglenatide (bound to immunoglobulin Fc fragments), and albiglutide (bound to albumin) [30–32]. These GLP-1 compounds degrade slowly, with half-lives of approximately 1 week, enabling a once-weekly injection [28]. Another GLP-1 RA, semaglutide, has a modified chemical structure that promotes binding to albumin, enabling a similar week-long half-life. Additionally, semaglutide was the first GLP-1 RA approved for oral administration [33].

Table 1 presents a summary of the characteristics of these GLP-1 RAs, including pharmacokinetics, dosing frequency, and administration.

**Table 1.** Characteristics of GLP-1 RAs.

| GLP-1 RAs | First Approved Date | Amino Acid Sequence | Elimination Half-Life | Administration Schedule | Phase III Clinical Trial Program | Reference |
|---|---|---|---|---|---|---|
| For subcutaneous injection | | | | | | |
| Exenatide | 2005 (USA); 2006 (Europe); | Exendin-4 | 3.3–4.0 h | Twice daily | AMIGO | [26] |
| Liraglutide | 2009 (Europe); 2010 (USA); | Mammalian GLP-1 | 12.6–14.3 h | Once daily | LEAD | [27] |
| Once-weekly exenatide | 2012 | Exendin-4 | 3.3–4.0 h | Once weekly | DURATION | [34] |
| Lixisenatide | 2013 (Europe); 2016 (USA); | Exendin-4 | 2.7–4.3 h | Once daily | GetGoal | [35] |
| Dulaglutide | 2014 | Mammalian GLP-1 | 4.7–5.5 days | Once weekly | AWARD | [31] |
| Albiglutide | 2014 (Europe); | Mammalian GLP-1 | 5.7–6.8 days | Once weekly | HARMONY | [36] |
| Semaglutide (SQ) | 2017 (USA); 2019 (Europe); | Mammalian GLP-1 | 5.7–6.7 days | Once weekly | SUSTAIN | [20] |
| For oral administration | | | | | | |
| Semaglutide (long-acting) | 2020 | Mammalian GLP-1 | 5.7–6.7 days | Once daily | PIONEER | [33] |

## 4. Reduction in Blood Glucose Levels and Weight

We identified eight trials evaluating the efficacy of GLP-1 RAs in reducing glucose levels and weight in patients with T2DM: DURATION-1, LEAD-6, DURATION-5, DURATION-6, HARMONY-7, AWARD-6, SUSTAIN-3, and SUSTAIN-10. The levels of HbA1c and weight reduction observed in patients taking the GLP-1 RA regimens in these trials are presented in Table 2.

**Table 2.** Comparison of associated reductions in blood glucose level and weight for various GLP-1 RAs.

| | Active Comparators | Change in HbA1c | Change in Weight | Reference |
|---|---|---|---|---|
| DURATION-1 | Exenatide 10 μg BID | −1.5 | −3.6 | |
| | Exenatide 2 mg QW | −1.9% | −3.7 | [26] |
| | *p* value | 0.0023 | 0.89 | |
| DURATION-5 | Exenatide 10 μg BID | −0.9 | −1.4 | |
| | Exenatide 2 mg QW | −1.6 | −2.3 | [37] |
| | *p* value | <0.0001 | <0.05 | |
| DURATION-6 | Exenatide 2 mg QW | −1.28 | −2.68 | |
| | Liraglutide 1.8 mg QD | −1.48 | −3.57 | [38] |
| | *p* value | 0.02 | 0.0005 | |
| LEAD-6 | Exenatide 10 μg BID | −0.79 | −2.87 kg | |
| | Liraglutide 1.8 mg QD | −1.12% | −3.24 kg | [39] |
| | *p* value | <0.0001 | 0.22 | |

Table 2. *Cont.*

|  | Active Comparators | Change in HbA1c | Change in Weight | Reference |
|---|---|---|---|---|
| HARMONY 7 | Albiglutide 50 mg QW | −0.78 | −0.64 | [40] |
|  | Liraglutide 1.8 mg QD | −0.99 | −2.16 |  |
|  | *p* value | 0.0846 | <0.0001 |  |
| AWARD-6 | Dulaglutide 1.5 mg QW | −1.42 | −2.90 | [41] |
|  | Liraglutide 1.8 mg QD | −1.36 | −3.61 |  |
|  | *p* value | <0.0001 | 0.011 |  |
| SUSTAIN-3 | Semaglutide 1.0 mg QW | −1.5 | −5.6 | [20] |
|  | Exenatide 2 mg QW | −0.9 | −1.9 |  |
|  | *p* value | <0.0001 | <0.0001 |  |
| SUSTAIN-10 | Semaglutide 1.0 mg QW | −1.7 | −5.8 | [42] |
|  | Liraglutide 1.2 mg QD | −1.0 | −1.9 |  |
|  | *p* value | <0.0001 | <0.0001 |  |

DURATION-1 was a 30-week randomized control trial that demonstrated that 2 mg of exenatide once weekly yielded superior outcomes in reducing HbA1c (−1.9% vs. −1.5%, $p = 0.0023$) but similar outcomes in weight reduction (−3.7 kg vs. −3.6 kg, $p = 0.89$) relative to 10 μg of exenatide twice daily [43]. These results invite comparison with DURATION-6, a 26-week, open-label, randomized, parallel-group study. In this trial, liraglutide (administered once weekly) was compared with exenatide (administered twice daily), with the primary endpoint being HbA1c change. Liraglutide was associated with a significantly greater reduction in HbA1c levels than exenatide (−1.48% vs. −1.28%, $p = 0.02$). In terms of body weight decrease, the liraglutide group was also superior to the exenatide once-weekly group (−3.57 kg vs. −2.68 kg, $p = 0.0005$) [39]. A separate trial, DURATION-5, was an open-label, randomized study comparing exenatide injected once weekly with exenatide injected twice daily over a period of 24 weeks. The main outcome measure was the changes in HbA1c levels. Exenatide administered once weekly was associated with significantly greater reductions in HbA1c levels than exenatide administered twice daily (−1.6% vs. −0.9%, $p < 0.0001$). In terms of weight loss, exenatide once weekly was associated with greater weight loss than exenatide twice daily (−2.3 kg vs. −1.4 kg, $p < 0.05$) [37]. Based on these trials, this study's analysis concluded that liraglutide administered once weekly was associated with significantly lower HbA1c levels and weight than 2 mg of exenatide once weekly and 10 ug of exenatide twice daily.

The LEAD-6 trial, a 26-week open-label, parallel-group, multinational study, compared the administration of 10 ug exenatide twice daily with 1.8 mg liraglutide once weekly. The primary outcome of the study was a change in HbA1c levels. Liraglutide reduced HbA1c significantly more than exenatide twice daily (−1.12% vs. −0.79%, $p < 0.0001$). Liraglutide was also associated with significantly more weight loss than exenatide 10 ug twice daily (−3.24 kg vs. −2.87 kg, $p = 0.0005$) [38]. Liraglutide was also studied in HARMONY-7, a 32-week, open-label, phase 3, noninferiority study comparing albiglutide once weekly with liraglutide once weekly. The primary endpoint was a change in HbA1c levels. The study found no significant difference between liraglutide and albiglutide once weekly (−0.99% vs. −0.78%, $p = 0.0846$) for this outcome. However, liraglutide was associated with more weight loss than albiglutide (−2.16 kg vs. −0.64 kg, $p < 0.0001$) [40]. Liraglutide also performed well in another trial, AWARD-6. In this phase 3, randomized, open-label, parallel-group study comparing 1.5 mg of dulaglutide with 1.8 mg of liraglutide with a primary outcome of noninferiority of dulaglutide with liraglutide with respect to change in HbA1c levels, dulaglutide and liraglutide were associated with reductions in HbA1c of −1.42% and −1.36%, respectively (noninferiority $p$ value < 0.0001), which met the noninferiority criteria

defined by the study. Liraglutide was associated with significantly greater weight loss than dulaglutide (−3.61 kg vs. −2.90 kg, *p* = 0.011) [41]. In summary, with respect to lowering HbA1c levels, liraglutide was just as effective as albiglutide, dulaglutide, and exenatide twice daily. Moreover, liraglutide was associated with significantly more weight loss than dulaglutide, albiglutide, and twice daily exenatide.

The administration of 1 mg of semaglutide once weekly was compared with the administration of 2 mg of exenatide once weekly in SUSTAIN-3, a 56-week, phase 3a, open-label, parallel-group, randomized controlled trial. The primary endpoint was a change in HbA1c levels. The results revealed that semaglutide reduced HbA1c significantly more than exenatide (−1.5% vs. −0.9%, *p* < 0.0001). Additionally, the semaglutide group experienced greater weight loss than the exenatide group (−5.6 kg vs. −1.9 kg, *p* < 0.0001) [20]. Semaglutide was further evaluated in SUSTAIN-10, a 30-week, phase 3b, open-label trial comparing 1 mg of semaglutide once weekly with 1.8 mg of liraglutide daily. The primary outcome was a change in HbA1c levels from baseline. Semaglutide was associated with significantly greater reductions in HbA1c levels than liraglutide (−1.7% vs. −1.0%, *p* < 0.0001). Moreover, semaglutide was associated with significantly greater weight loss than liraglutide (−5.8 kg vs. −1.9 kg, *p* < 0.0001) [42]. In summary, 1 mg of semaglutide administered weekly was more effective than 2 mg of exenatide administered once weekly and 1.8 mg of liraglutide administered once daily.

After comprehensively reviewing the data on these GLP-1 RAs, we observed that in terms of glycemic control, the GLP-1 RAs could be ranked in descending order of effectiveness as follows: 1 mg of semaglutide, 1.8 mg of liraglutide, 1.5 mg of dulaglutide, 50 mg of albiglutide, 2 mg weekly of exenatide, and 10 μg twice daily of exenatide. The same relative order of superiority was observed in terms of associated weight loss. However, more and larger comparison trials are required to determine the efficacy of these various GLP-1 RAs.

## 5. Cardiovascular Effects

GLP-1 RAs yield improvements in cardiovascular (CV) outcomes due to their insulinotropic blood pressure reduction and weight-lowering action [44–46]. Moreover, in rodents, GLP-1 RAs increased cardiomyocyte survival by inhibiting apoptosis, improving regional and global cardiac output following injury or heart failure, and ameliorating endothelial dysfunction [47–49].

According to the American Diabetes Association guidelines from 2023, GLP-1 RAs offer several CV benefits to patients with T2DM and ASCVDs. According to them, Dulaglutide, liraglutide, and semaglutide can prevent major adverse cardiovascular events (MACEs), whereas exenatide and lixisenatide had no effect on the occurrence of MACEs [5]. Accordingly, the European Society of Cardiology guidelines from 2023 recommend sodium-glucose cotransporter 2 inhibitors (SGLT-2is) and GLP-1 Ras as a preferred glucose-lowering therapy for patients with T2DM and ASCVD [50].

Table 3 presents the basic characteristics of the trials and their primary composite cardiovascular (CV) outcomes; these trials compared GLP-1 RAs against placebos. With regard to three-point MACEs (CV-related death, myocardial infarction, and stroke), liraglutide, semaglutide, albiglutide, and dulaglutide significantly reduced the risks of all three MACES. Exenatide and semaglutide (oral) reduced MACE risk but not significantly so. Moreover, in the ELIXA study, lixisenatide did not reduce MACE risk. Compared with placebos, liraglutide and oral semaglutide significantly reduced the risk of CV-related death. By contrast, injection of dulaglutide, albiglutide, lixisenatide, exenatide, and semaglutide did not significantly reduce the risk of CV-related death. Myocardial infarction risk was significantly reduced by liraglutide and albiglutide; non-significantly reduced by dulaglutide, exenatide, lixisenatide, oral semaglutide, and semaglutide injection; and not reduced by lixienatide or oral semaglutide. Stroke risk was significantly reduced by only dulaglutide and non-significantly reduced by all other GLP-1 RAs except lixisenatide. Table 3 lists the individual hazard ratios for three-point MACEs in each trial.

Table 3. Comparison of GLP-1 RAs with respect to cardiovascular outcomes.

| Agent | Study | Median Follow-up | Prior CVD% | Primary Composite CV Outcome HR | *p* Value | Cardiovascular Death HR | *p* Value | Fatal or Nonfatal Myocardial Infarction HR | *p* Value | Fatal or Nonfatal Stroke HR | *p* Value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Efpeglenatide | AMPLITUDE-O | 1.81 | 89.6 | 0.73 | 0.007 | 0.72 | 0.07 | 0.75 | 0.09 | 0.74 | 0.19 |
| Semaglutide(oral) | PIONEER-6 | 1.3 | 85 | 0.79 | 0.17 | 0.49 | 0.021 | 1.04 | 0.49 | 0.76 | 0.43 |
| Semaglutide | SUSTAIN-6 | 2.1 | 59 | 0.74 | 0.02 | 0.98 | 0.92 | 0.81 | 0.26 | 0.65 | 0.066 |
| Albiglutide | Harmony | 1.6 | 100 | 0.78 | 0.0006 | 0.93 | 0.58 | 0.75 | 0.003 | 0.86 | 0.3 |
| Dulaglutide | REWIND | 5.4 | 32 | 0.88 | 0.026 | 0.91 | 0.21 | 0.96 | 0.63 | 0.76 | 0.01 |
| Lixisenatide | ELIXA | 2.1 | 100 | 1.02 | 0.81 | 0.98 | 0.85 | 1.03 | 0.71 | 1.12 | 0.54 |
| Liraglutide | LEADER | 3.8 | 81 | 0.87 | 0.01 | 0.78 | 0.007 | 0.86 | 0.046 | 0.86 | 0.16 |
| Exenatide | EXSCEL | 3.2 | 73 | 0.91 | 0.06 | 0.88 | 0.096 | 0.97 | 0.62 | 0.85 | 0.095 |

In the EXSCEL trial, once-weekly exenatide exhibited significant noninferiority to placebo in three-point MACEs (hazard ratio: 0.91; 95% confidence interval [CI]: 0.83–1.00). However, with respect to the individual components of three-point MACES—cardiovascular-related death, myocardial infarction, and stroke—once-weekly exenatide did not differ significantly compared with placebos [51]. In the LEADER trial, liraglutide had significantly fewer three-point MACEs (hazard ratio: 0.87; 95% CI: 0.78–0.97; *p* < 0.001 for noninferiority; *p* = 0.01 for superiority). Liraglutide was also significantly superior to placebos with respect to CV-related death (hazard ratio: 0.78; 95% CI: 0.66–0.93; *p* = 0.007) and myocardial infarction (hazard ratio: 0.86; 95% CI: 0.73–1.00; *p* = 0.046). With respect to stroke, liraglutide was similar to placebos [52]. In the SUSTAIN-6 trial, subcutaneous semaglutide was associated with significantly fewer three-point MACEs (hazard ratio: 0.74; 95% CI: 0.58–0.95; *p* < 0.001 for noninferiority; *p* = 0.02 for superiority). However, the differences with respect to individual MACES were not significant compared with placebos [53]. Additionally, in the ELIXA trial, the lixisenatide group had significant noninferiority to the placebo group with respect to three-point MACEs (*p* < 0.001) but did not exhibit superiority (*p* = 0.81) (hazard ratio: 1.02; 95% CI: 0.89–1.17). No significant difference was observed between the two groups with respect to the individual MACEs [54]. Furthermore, in the Harmony Outcomes trial, albiglutide exhibited significant superiority to placebo with respect to three-point MACEs (hazard ratio: 0.78, 95% CI: 0.68–0.90) (*p* < 0.0001 for noninferiority; *p* = 0.0006 for superiority). Albiglutide was also associated with significantly improved outcomes with respect to myocardial infarction (hazard ratio: 0.75; 95% CI: 0.61–0.90; *p* = 0.003). With respect to stroke and CV-related death, no significant differences were observed [55]. Additionally, in the REWIND trial, dulaglutide was associated with significantly fewer three-point MACEs (hazard ratio: 0.88: 95% CI: 0.79–0.99; *p* = 0.026). Moreover, in terms of stroke, dulaglutide exhibited significant superiority to placebos (HR: 0.76, 95% CI: 0.62–0.94; *p* = 0.010). However, with respect to myocardial infarction and CV-related death, no significant differences were observed [56]. Furthermore, in the PIONEER-6 trial, oral semaglutide exhibited significant noninferiority to placebo with respect to three-point MACEs (hazard ratio: 0.79; 95% CI: 0.57–1.11; *p* < 0.001 for noninferiority). Oral semaglutide was also associated with a significantly lower risk of CV-related deaths (hazard ratio: 0.49; 95% CI: 0.27–0.92); *p* = 0.021). However, with respect to stroke and myocardial infarction, no significant differences were observed [57]. In another trial (AMPLITUDE-O), efpeglenatide was associated with significantly fewer three-point MACEs (hazard ratio: 0.73; 95% CI: 0.58–0.92; *p* < 0.001 for noninferiority; *p* = 0.007 for superiority). The results for other components of the primary outcome pertained to CV-related death (hazard ratio: 0.49; 95% CI: 0.27–0.92, *p* = 0.07), nonfatal myocardial infarction (hazard ratio: 1.18; 95% CI: 0.73–1.90, *p* = 0.09), and nonfatal stroke (hazard ratio: 0.74; 95% CI: 0.35–1.57 *p* = 0.19) [56].

A pooled meta-analysis of the ELIXA, LEADER, SUSTAIN-6, EXSCEL, Harmony Outcomes, REWIND, PIONEER 6, and AMPLITUDE-O trials conducted by Lancet Diabetes Endocrinol 2021 revealed that GLP-1 RAs resulted in a 14% relative risk reduction for three-

point MACES compared with placebos (hazard ratio: 0.86, 95% CI: 0.80–0.93; $p < 0.0001$). In individual measures of the three-point MACEs, GLP-1 RAs contributed to a reduction in risk of death from CV causes (hazard ratio: 0.87; 95% CI: 0.80–0.94; $p = 0.0010$), myocardial infarction (hazard ratio: 0.90; 95% CI: 0.83–0.98; $p = 0.020$), and stroke (hazard ratio: 0.83; 95% CI: 0.76–0.92; $p = 0.0002$). Moreover, when ELIXA was excluded from the meta-analysis, overall CV benefits of GLP-1 RAs would modestly increase. These results provide further evidence that GLP-1 RAs reduce the occurrence of three-point MACEs and each of their components.

An analysis of these randomized controlled trials (ELIXA, LEADER, SUSTAIN-6, EXSCEL, Harmony Outcomes, REWIND, PIONEER 6, and AMPLITUDE-O) reveals that liraglutide, dulaglutide, albiglutide, semaglutide, and efpeglenatide were associated with statistically significant reductions in three-point MACEs. With respect to individual MACEs, liraglutide and oral semaglutide were significantly associated with reductions in CV-related deaths, aliraglutide and albiglutide were associated with significant reductions in the incidence of myocardial infarctions, and only liraglutide was associated with a significant reduction in the incidence of stroke. One explanation for these findings is that these typical GLP-1 RAs exhibit the ''classic effect'' for their medication type and thus had similar influences on CV outcomes.

## 6. Heart Failure

With respect to heart failure (HF), many hypoglycemic agents, such as thiazolidine-diones, exert a strong influence on the risk of developing HF requiring hospitalization. GLP-1 RAs may also affect left ventricular ejection fraction (LVEF) and HF with preserved ejection fraction (HFpEF). The results of meta-analyses of phase-II/III trials (for exenatide, albiglutide, dulaglutide, and liraglutide) revealed that GLP-1RAs were not associated with increased risk of hospitalization for HF, indicating their safety in patients who also have CVD. Three large prospective cardiovascular outcome trials (ELIXA [on lixisenatide], LEADER [on liraglutide], and SUSTAIN-6 [on semaglutide]) have further demonstrated the low risk of HF associated with GLP-1 RAs [31]. Moreover, the STEP-HFpEF trial indicated that semaglutide improved physical functioning (as assessed by 6-min walk distance), preserved HFpEF, and reduced weight in patients with HF and obesity [58]. However, randomized controlled trials of liraglutide (LIVE and FIGHT) and Exenatide (EXSCEL) indicated that these medications increased the risk of hospitalization in patients with HF with a reduced ejection fraction [59–61]. The increased risk of hospitalization observed in these trials may be attributable to differences in these medications' effects on LVEF. LVEF was observed to be 57%, 33%, and 27% in the STEP-HFpEF, LIVE, and FIGHT trials, respectively. Another trial (the SELECT trial) targeted patients with obesity but without T2DM. This trial indicated that semaglutide was significantly superior to placebos in reducing the occurrence of MACEs (hazard ratio: 0.80; 95% CI: 0.72–0.90; $p < 0.001$) [62].

In conclusion, semaglutide reduced HF and the risk of CV events in non-diabetic patients with obesity. However, liraglutide and exenatide increased hospitalization in diabetic patients with HF and a reduced ejection fraction. This meta-analysis found no evidence indicating the effects of other GLP-1 RAs on HF in patients with T2DM or obesity.

## 7. Renal Effects

GLP-1 RAs may improve kidney function through direct or indirect mechanisms. One study suggested that the signaling pathway activated by the binding of GLP-1 and GLP-1 receptors results in the phosphorylation of PKA consensus sites at the NHE3 COOH-terminal region. Once the PKA consensus sites have been phosphorylated, sodium, bicarbonate, and water reabsorption are decreased through the inhibition of NHE3-mediated Na+/H+ exchange in the proximal tubule [9]. Additionally, studies have suggested that the observed renal benefits of GLP-1 RAs stem from indirect interactions between the nervous system [63] and the renin–angiotensin system (RAS) [64], in addition to the regulation of atrial natriuretic peptides (ANPs) [65].

According to the American Diabetes Association guidelines from 2023, GLP-1 RAs, especially liraglutide, dulaglutide, and semaglutide, were associated with beneficial renal outcomes in CV outcome trials; these renal benefits were driven by new onset or persistent macroalbuminuria outcomes [5].

Evidence for the renal benefits of GLP-1 RAs was available for semaglutide, dulaglutide, and liraglutide. We found no complete data on the subgroups of composite renal outcomes for other GLP-1 RAs, (exenatide, lixisenatide, albiglutide, efpeglenatide, and oral semaglutide). Available data are presented in Table 4. With regard to liraglutide, the LEADER trial indicated that this GLP-1 RA was associated with lower rates of the development and progression of diabetic kidney disease than placebos. These results were based on the secondary renal outcomes of the trial, which indicated fewer participants in the liraglutide group than in the placebo group (268 of 4668 patients vs. 337 of 4672; hazard ratio: 0.78; 95% CI: 0.67–0.92; $p = 0.003$) Liraglutide was also associated with significant decreases in the incidence of macroalbuminuria (161 of 4668 patients vs. 215 of 4672; hazard ratio: 0.74; 95% CI: 0.61 to 0.91; $p = 0.004$). However, with respect to the sustained doubling of serum creatinine and the requirement for continuous renal replacement therapy, liraglutide was not associated with significant reductions in these outcomes [52,66]. With regard to the renal outcomes of dulaglutide in patients with T2DM, an exploratory analysis of the REWIND randomized placebo-controlled trial revealed that fewer negative composite renal outcomes were associated with long-term use of dulaglutide compared with placebo (hazard ratio: 0.85; 95% CI: 0.77–0.93; $p = 0.0004$). Moreover, dulaglutide was associated with a reduction in the occurrence of macroalbuminuria (hazard ratio: 0.77; 95% CI: 0.68–0.87; $p < 0.0001$). Additionally, no significant difference was observed between dulaglutide and placebos in the requirement for continuous renal replacement therapy [67]. In the SUSTAIN-6 study, semaglutide was also associated with reduced incidence rates of new or worsening nephropathy (hazard ratio: 0.64; 95% CI: 0.46–0.88; $p = 0.005$). Furthermore, semaglutide was associated with a significantly reduced occurrence of macroalbuminuria (hazard ratio: 0.54; 95% CI: 0.37–0.77; $p = 0.001$). Finally, no significant difference was observed between semaglutide and placebo regarding the requirement for continuous renal replacement therapy [53].

**Table 4.** Comparisons of GLP-1 RAs with respect to renal outcomes.

| Agent | Semaglutide | Dulaglutide | Liraglutide |
|---|---|---|---|
| Study | SUSTAIN-6 | REWIND | LEADER |
| Median follow-up | 2.1 | 5.4 | 3.8 |
| Composite renal outcome HR | 0.64 | 0.85 | 0.78 |
| *p* value | 0.005 | 0.0004 | 0.003 |
| New onset of macroalbuminuria HR | 0.54 | 0.77 | 0.74 |
| *p* value | 0.001 | <0.0001 | 0.004 |
| Sustained doubling of serum creatinine HR | 1.28 | 0.89 | 0.89 |
| *p* value | 0.48 | 0.07 | 0.43 |
| Need for continuous renal replacement therapy HR | 0.91 | 0.75 | 0.87 |
| *p* value | 0.8 | 0.4 | 0.4 |

In conclusion, semaglutide, liraglutide, and duraglutide were associated with benefits to composite renal outcomes, particularly a decreased incidence of macroalbuminuria, but were not significantly associated with other renal benefits, such as reduced estimated glomerular filtration rate (eGFR) deterioration or a reduced requirement for renal replacement therapy.

## 8. Conclusions

The GLP-1 RAs reviewed in this meta-analysis were associated with safety, weight loss, glucose reduction, CV outcomes, HF, and renal outcomes. In descending order of benefit to glycemic control, semaglutide was statistically superior to liraglutide, dulaglutide, albiglutide, 2 mg of exenatide weekly, and 10 μg of exenatide twice daily. This same order of superiority was observed with respect to associated weight loss. Moreover, these GLP-1 RAs all improved CV outcomes overall but had different associations with the incidence of individual MACES. In particular, oral 14 mg daily semaglutide and 1.8 mg daily injected liraglutide were associated with a reduced risk of CV death, although this benefit was not observed for the other GLP-1 RAs. Semaglutide was associated with superior outcomes for HF and other CV outcomes in non-diabetic patients with obesity; by contrast, liraglutide was associated with worse HF outcomes in patients with diabetes with a reduced ejection fraction. In terms of benefits to kidney function, semaglutide, dulaglutide, and liraglutide were associated with superior composite renal outcomes, reducing the occurrence of new or persistent macroalbuminuria; however, these GLP-1 RAs were not associated with benefits to the occurrence of eGFR deterioration or the requirement for continuous renal replacement therapy. Finally, GLP-1 RAs may provide additional benefits to patients with obesity or diabetes.

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