# ORIGINAL ARTICLE

# Evaluation of Prevalence, Management and Recording of Risk Factors in Outpatient Patients with Coronary Artery Disease

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#### **Abstract**

**Background**: Coronary artery disease (CAD) is the leading cause of mortality worldwide. Adequate management of risk factors is directly correlated with better outcomes.

**Objectives**: This study aims to identify risk factors and evaluate the indicated medical treatment (both pharmacological and non-pharmacological) in patients with CAD.

**Methods**: Epidemiological data were collected from electronic medical records of patients treated at Cardiology outpatient clinics of a Tertiary Hospital and a descriptive analysis of the data was performed.

Results: Patients with documented CAD (n = 230) were selected for the study, with a predominance of white (60%) men (62%), most of whom were monitored following an Acute Coronary Syndrome (ACS) (63.9%). High prevalence and irregular control of risk factors such as smoking, hypertension, dyslipidemia, diabetes, obesity, and sedentary lifestyle were observed. Target blood pressure was achieved in 52.6% of patients, low-density lipoprotein (LDL) in 5.7%, and HbA1c in 50.8%, despite appropriate prescriptions for most patients – 100% were prescribed statins, and over 90% received antiplatelet agents, angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers, and beta-blockers. Few patients were encouraged to quit smoking and engage in physical activities.

**Conclusion**: Significant recording errors were noted in the medical records, along with inadequate control of CAD-related risk factors. Improvements in both recording and management of these risk factors are necessary to enhance CAD outcomes. The study utilized a straightforward and cost-effective model, which effectively mapped the local population profile and managed CAD risk factors.

Keywords: Cardiovascular Diseases; Heart Disease Risk Factors; Coronary Artery Disease; Secondary Prevention.

## Introduction

Cardiovascular diseases (CVDs) and coronary artery disease (CAD) are still the leading causes of death both globally and in Brazil. The World Health Organization (WHO) estimated that ischemic heart disease and strokes, combined, caused more than 25% of deaths worldwide in 2019.¹ In Brazil, CAD remains the leading cause of death, with nearly 2% prevalence in the population, being responsible for 12% of deaths in 2019.² In comparison, CVD accounts for approximately 30% of death causes.³

Identifying individuals with atherosclerosis is crucial for establishing treatment and secondary prevention measures by assessing the risk of atherosclerotic disease. CAD probability results from variables such as the characterization of chest pain, sex, comorbidity, and age of the patient. Factors such as smoking (minimum of 25 pack-years), total cholesterol (> 250 mg/dL), and fasting blood glucose (> 140 mg/dL) also increase the risk of CAD.<sup>3</sup>

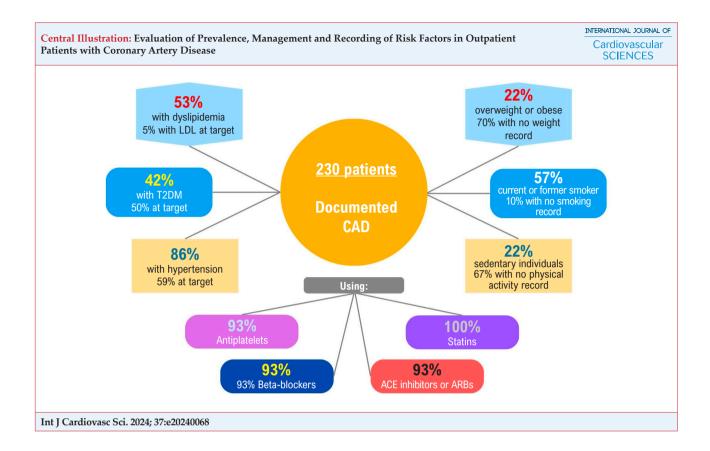
It is understood that cardiovascular risk factors such as systemic arterial hypertension (SAH), diabetes mellitus, dyslipidemia, smoking and obesity are potentially

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modifiable. Their lack of control is associated with worse cardiovascular outcomes and higher mortality; therefore, the management of these factors is essential in terms of prevention.<sup>3-5</sup> Likewise, the control of risk factors and the drug treatment of CAD bring clear benefits in reducing mortality rates.<sup>3,4</sup>

This study was designed in line with the Survey of Risk Factor Management (SURF) trial<sup>6,7</sup> and EUROASPIRE<sup>8,9</sup> studies, which collect data on demographics, risk factors, and comorbidity management in CAD patients. While the SURF study is ongoing, previous publications have reported data from a pilot project and an audit involving 11 European countries,<sup>7</sup> similar to the data collected in this study.

Therefore, Thus, this research aimed to evaluate the epidemiological status and prevalence of risk factors in CAD patients. Additionally, the study aimed to audit comorbidity records and assess the achievement of targets related to CAD risk factors, as well as to evaluate the quality of risk factor management and treatment for the selected patients.

# Methodology

This is an epidemiological, observational and crosssectional study. Using a data collection form, patients treated at the Cardiology outpatient clinics of a Tertiary Hospital were selected. The inclusion criteria were patients treated during 2022, over 18 years of age, of both sexes, with CAD proven by one of the following criteria: a) Acute Coronary Syndrome (ACS); b) Stable Angina with typical alteration of Electrocardiogram, exercise test suggesting ischemia, positive scintigraphy for ischemia or lesion greater than or equal to 70% in one or more coronary arteries, as evidenced by coronary angiotomography or coronary cineangiography.

The information was collected by consulting the patients' electronic medical records without direct contact or new interference, all of which are presented in Table 1. The data were compiled into a Microsoft Excel spreadsheet and analyzed by calculating percentages, averages, and standard deviations.

The blood pressure and lipid profile variables were classified according to the targets established in the Cardiovascular Prevention Guideline.<sup>4</sup> Thus, the blood pressure target adopted was systolic blood pressure (SBP) < 130mmHg and diastolic blood pressure (DBP) < 80mmHg. There is also a recommendation to avoid SBP < 120mmHg and DBP < 70mmHg, especially DBP < 60mmHg, due to the risk of coronary hypoperfusion. Regarding the lipid profile,

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#### Identification data Laboratory data collected in the last year

Age Total cholesterol

Sex HDL and LDL cholesterol fractions

Ethnicity Triglycerides

Education level Fasting blood glucose

Hemoglobin A1c

#### Main diagnosis Record of non-pharmacological measurements

Chronic coronary syndrome

Guidance on smoking cessation

ACS

Guidance on engaging in physical activity

#### Risk factors Treatment(s) established

History of smoking Degree of physical activity

Degree of physical activity Percutaneous coronary intervention
Family history Myocardial revascularization surgery
History of hypertension Exclusive clinical treatment

History of dyslipidemia Need for hospitalization in the last year due to coronary disease

History of diabetes (types 1 or 2)

#### Medical prescription Information from the last consultation

Antiplatelet agents

Statins

Others Lipid reducers

Beta-blockers

ACE inhibitors

Angiotensin II receptor blockers (with or without neprilysin inhibitor)

Calcium channel blockers

Diuretics
Other antihypertensives

Nitrates

Anti-ischemics (Trimetazidine)

Oral hypoglycemic agents

Insulins

Last visit ≤ 3 months ago? Systolic and DBP Heart rate

Body weight and height Abdominal circumference

HDL: high-density lipoproteins; LDL: low-density lipoproteins; ACE: angiotensin-converting enzyme; DBP: diastolic blood pressure; ACS: acute coronary syndrome.

the target was considered to be total cholesterol < 190, HDL > 40, trigly cerides < 150, and LDL < 50.

For targets related to Diabetes, the Brazilian Diabetes Guidelines<sup>10</sup> were used, establishing it at HbA1c < 7.0% for individuals in any age group, aiming to prevent long-term macrovascular complications as long as there is no risk of severe and frequent hypoglycemia. For elderly individuals with diabetes and CVD, less strict glycemic targets may be considered, such as an HbA1c between 7.0 and 7.5%. The diagnosis of Metabolic Syndrome was established based on the NCEP-ATP III criteria, as cited by the I Brazilian Guideline for Diagnosis and Treatment of Metabolic Syndrome.<sup>11</sup>

Body mass index (BMI) was calculated according to the definitions of the WHO and the Brazilian Obesity Guidelines, <sup>12</sup> which classify Underweight for BMI <  $18.5 \text{ kg/m}^2$ , Normal Weight from  $18.5 \text{ to } 24.9 \text{ kg/m}^2$ , Overweight from  $25 \text{ to } 29.9 \text{ kg/m}^2$ , Obesity grade I from  $30 \text{ to } 34.9 \text{ kg/m}^2$ , Obesity grade II from  $35 \text{ to } 39.9 \text{ kg/m}^2$ , and Severe Obesity  $\geq 40 \text{ kg/m}^2$ . The European and North American Guidelines for the Prevention of CVDs established targets very similar to those of the Brazilian guidelines. Therefore, these were used to define the targets in this study.

The collected data allowed for the determination of the sample's epidemiological profile, including the

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The project for this research was submitted to the local Ethics Committee and approved under number CAAE 61589422.4.0000.0107, in compliance with the terms of resolution 466/2012 of the Brazilian National Health Council.

## Statistical analysis

The collected information was organized and analyzed using Microsoft Excel 2016, employing descriptive statistical methods. Quantitative variables were described with means and standard deviations, with the normality of the sample distribution tested using the Shapiro-Wilk test and calculations performed using Python software. Statistically significant values were p < 0.05. Categorical variables were represented by the absolute value (n) and their respective frequency (%) in relation to the total sample.

#### Results

#### Epidemiology, Risk Factors, And Management

Based on the inclusion criteria, 230 patient records were analyzed (n = 230). All epidemiological and demographic data are presented in Table 2.

Table 3 presents data related to the main diagnosis, interventions and monitoring. Most patients continue outpatient follow-up after ACS (63.9%), and the predominant treatment among the population studied was Percutaneous Coronary Intervention (53.8%). Of the entire sample, 42.4% of the patients evaluated were hospitalized in the last year due to CAD.

Table 4 summarizes the prevalence of CAD risk factors. Within the sample studied, more than 22% of patients had a BMI equal to or greater than 25 kg/m². However, metabolic syndrome was diagnosed in only one patient, the only one with a record of abdominal circumference in the medical records.

The majority of the study population (67%) had an outpatient medical consultation in the last three months. According to the physical examination data from the last recorded consultation, an average SBP of 124 mmHg

Table 2 – Epidemiological and demographic data			
Sex	%		
Male	142 (61.7)		
Female	88 (38.3)		
Ethnicity			
White	139 (60.4)		
Mixed-race	39 (17)		
Black	2 (0.9)		
Not recorded	50 (21.7)		
Education level			
ncomplete elementary school	57 (24.8)		
Complete elementary school	60 (26.1)		
Complete high school	16 (7)		
College or higher	6 (2.6)		
Not recorded	91 (39.6)		

and DBP of 75 mmHg were found. Table 5 presents data related to blood pressure control.

Regarding laboratory tests, approximately 75% of patients had laboratory results collected in the last three months. The lipid profile was measured in 74.7% of the patients included (n = 172) while fasting blood glucose and Hemoglobin A1c were measured in only 66.3% of the diabetic patients (n = 65). Among the diabetic patients, the mean blood glucose was 131 ( $\pm$  50.8), and approximately 49.2% had Hemoglobin A1c values above those expected for patients with CAD. Among the patients with no previous diabetes diagnosis, none met the laboratory diagnostic criteria during routine exams. Table 6 summarizes the data related to the laboratory targets.

Regarding treatment, Table 7 presents data on the treatment instituted, including the percentage of patients receiving each medication class, as well as non-pharmacological guidelines. The analysis revealed that nearly all patients were prescribed antiplatelet medications (93.9%), while those not receiving antiplatelet drugs were on oral anticoagulants for atrial fibrillation or flutter. Furthermore, among the 15 patients not prescribed beta-blockers, 80% had absolute contraindications for their use.

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# Assessment of the Quality of Medical Records

Data collection from medical records provided insights into the epidemiological profile and management of coronary disease within the studied population, as well as an evaluation of the quality of medical record documentation at the Cardiology outpatient clinics. Regarding patient identification data from initial registration, over 20% of patients lacked ethnicity information, and 40% of records did not include educational level.

Additionally, several significant gaps were identified in the medical records. Regarding risk factors, the smoking history was absent in 10% of records, and the patient's level of physical activity was documented in only about 32% of records. Furthermore, approximately 73% of the medical records had no record of family history.

Height and weight data were found in 29.6% and 41.3% of the medical records, respectively. For 70.4% of patients, BMI could not be calculated due to a lack of data. Abdominal circumference was measured in only one patient out of the total 230 under analysis. There were no laboratory tests from the last year, including a lipid profile, for 25.2% of patients, and 32.7% of diabetic patients did not have their HbA1c levels measured. Regarding treatment, there was a record of medical prescriptions and/or medications for continuous use for all patients. However, non-pharmacological measures, such as guidance on performing

Smoking	
moker	32 (13.9)
ormer smoker	100 (43.5)
Never smoked	75 (32.6)
Not recorded	23 (10)
Comorbidities	
АН	199 (86.5)
Pyslipidemia	122 (53)
ype I DM	0 (0)
ype II DM	98 (42.6)
Obesity and metabolic syndrome	
Normal weight	16 (7)
Overweight	28 (12.2)
Desity	24 (10.5)
lot recorded	162 (70.4)
Metabolic syndrome	1 (0.4)
Exercise	
edentary <sup>‡</sup>	51 (22.2)
/Ioderate§	21 (9.1)
evere <sup>//</sup>	3 (1.3)
Not recorded	155 (67.4)
Family history	
ignificant for CVD	29 (12.6)
Not significant	31 (13.5)
Not recorded	170 (73.9)

physical activities, were not recorded in 67.4% of medical records. Central Illustration summarizes the main findings of this article.

"Includes activity higher than the recommended level.

#### Discussion

This article replicated the model proposed by the multicenter study SURF,<sup>6,7</sup> applying data collection form

Table 5 – Blood Pressure	Control		
SBP		DBP	
SBP < 120mmHg	89 (38.7)	DBP < 70mmHg	52 (22.6)
SBP ≥ 130mmHg	93 (40.4)	DBP≥80mmHg	97 (42.2)
DBP on target	48 (20.9)	DBP on target	81 (35.2)
BP < 130x80mmHg	137 (59.6)	SBP and DBP at target <sup>§</sup>	23 (10)
Mean SBP	124 (±21.7) <i>p</i> = 0.003	Mean DBP	75 (± 12.6) P = 0.002

Values represent mean (standard deviation) or number of records (%).

§BP strictly at target, between 120x70mmHg and 130x80mmHg, according to the Brazilian Prevention Guideline.

SBP: systolic blood pressure; DBP: diastolic blood pressure; BP: blood pressure.

Table 6 - Control of Dyslipidemia and Diabetes

Lipid profile measured	Mean	At target		
Total cholesterol	159 (±40.4) <i>p</i> =0.001	134 (58.3)		
LDL	86 (±32.8) <i>p</i> =0.001	13 (5.7)		
HDL	47 (±14.9) <i>p</i> =0.001	118 (51.3)		
Triglycerides	153 (±91.5) <i>p</i> =0.001	104 (45.2)		
HbA1c measured (in diabetic patients)				
HbA1c	7.1 (±2.0) <i>p</i> =0.001	33 (50.8)		

Values represent mean and standard deviation or number of records (%). HbA1c: Hemoglobin A1c; HDL: high-density lipoproteins; LDL: low-density lipoproteins.

to the medical records of patients with CAD. This model is easily replicated in different centers, thus facilitating the mapping and management of risk factors in outpatients, in addition to allowing the auditing of data from the service itself and assessing the quality of medical records information. In this sample, the application process proved to be straightforward and efficient, similar to the SURF<sup>6</sup> study, as it involved only reviewing medical records without needing to contact patients for additional consultations.

Given the same model, this study can be compared to the SURF<sup>6,7</sup> studies, as well as to similar studies found in the literature, such as the EUROASPIRE.<sup>8,9</sup> The identified demographic and epidemiological profile reflects the reality of a public tertiary hospital in southern Brazil, with a predominance of a white population with low levels of education since more than half of the sample had completed elementary school at most. This profile contrasts with the population studied in SURF, which included private patients – 12% in the European sample and 37% in the Asian sample – and also had a higher level of education.

The mean age found was similar to published SURF studies, as well as to a Brazilian study using the same model.<sup>15</sup> The age range in these studies is slightly higher than the average of the EUROASPIRE studies and another national study carried out in Intensive Care Units.<sup>16</sup> The proportion of men in our sample was lower than that found in the SURF and Mertins et al.<sup>16</sup> studies (61.7% vs. 70.8 and 81.2%, respectively).

The main reason for outpatient follow-up was ACS, similar to the SURF studies<sup>67</sup> (63.9% of cases), with a predominance of treatment by PCI (58.3%), and almost half of the patients (42.4%) were hospitalized in the last year due to CAD. This finding reflects the Hospital's profile, which is currently the reference in ACS care in the region for the Brazilian Unified Health System. International studies<sup>67</sup> have found a 29% to 50% hospitalization rate in the last year due to CAD.

Regarding follow-up, approximately 67% of patients had been seen in the past 3 months, and nearly 75% had recent laboratory test results (including lipid profiles, fasting blood glucose, and hemoglobin A1c from the past year) in their medical records. This situation reflects the limitations of the public health system and the overcrowding of specialty outpatient clinics like ours. However, it indicates that medical care and management of comorbidities are below expectations due to infrequent consultations and a lack of diagnostic tests. This hinders effective interventions for reducing cardiovascular risk, such as managing

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Table 7 – Treatment of CAD and risk factors					
Pharmacological treatment	%		%		
Antiplatelet agents	216 (93.9)	Statins	230 (100)		
ACE inhibitors	128 (55.7)	ARB	87 (37.8)		
ВВ	215 (93.5)	Contraindication to BB‡	12 (80)		
Diuretics	152 (66.1)	Calcium channel blockers	52 (22.6)		
Other antihypertensives	15 (6.5)	Other lipid-lowering agents§	68 (29.6)		
Nitrates	20 (8.7)	Trimetazidine	40 (17.4)		
Oral hypoglycemic agents	89 (90.8)	Insulins	21 (21.4)		
Non-pharmacological measures					
Smoking cessation	15 (27.3)				
Exercise	20 (9.7)				

Values represent the number of records (%).

ACE inhibitors: Angiotensin-converting enzyme inhibitors; ARB: Angiotensin II receptor blockers; BB: Beta-blockers.

Table 8 – Comparison between prevalence of risk factors							
Risk factors	This study	SURF <sup>7</sup> 2016	SURF <sup>6</sup> 2014	EUROº III	Mertins et al. <sup>16</sup> 2013	Silveira et al. <sup>15</sup> 2018	INTER HEART <sup>17</sup>
Smoking	13.9	16.2	14-15	17.2	41.7	6.6	45.1
Sedentary lifestyle	22.2	46.6	41-45	69.8	91.7	74.4	85.7
FH positive	12.6	-	-	-	43.4	-	-
SAH	86.5	74.5	70-71	56	63.8	83.3	39
Dyslipidemia	53	67.6	61-77	76.5	23	62.2	49.2
DM	42.6	34.5	22-32	24.6	20.8	34.4	18.4
Overweight and obesity	22.7	72.5	59-78	81.8	68.9	64.4	30.2*

Source: own authorship; SURF (2016); SURF (2014); EURO III (2009)

Values represent the prevalence (%).

\*INTERHEART used abdominal obesity, not BMI, as a reference.

SAH: systemic arterial hypertension; DM: diabetes mellitus; FH: familial history; SURF: SUrvey of Risk Factor Management trial.

underdiagnosed diabetes or prescribing statins for potential dyslipidemia.

Similar to other studies, a high prevalence of modifiable risk factors associated with increased cardiovascular risk is evident. Table 8 compares the prevalence of risk factors between national and international multicenter studies and the present study.

The Brazilian Guideline for Cardiovascular Prevention4 advocates for smoking cessation counseling and intensive treatment (grade IA recommendation), as well as counseling for physical activity and weight loss, emphasizing moderate to vigorous exercise due to its proven reduction in cardiovascular risk (grade IA recommendation). Additionally, it recommends intensive management of

<sup>&</sup>lt;sup>‡</sup>Absolute contraindication among patients who were not using beta blockers.

<sup>§</sup>Only Ezetimibe was verified in the sample.

dyslipidemia, hypertension, and diabetes to mitigate cardiovascular event risk. The INTERHEART<sup>17</sup> study found a clear link between individual and cumulative risk factors and a higher incidence of acute myocardial infarction, with current smoking and dyslipidemia being the primary factors. This study suggests that controlling modifiable risk factors could prevent most myocardial infarctions.

In this study, only 5.7% of patients had an LDL level < 50, and just 24.8% had an LDL level < 70. These LDL control rates are significantly lower than those reported in similar studies. European and Asian studies (SURF and EUROASPIRE<sup>6-9</sup>) reported LDL within the target for 48.9% to 66% of patients, even using a stricter LDL target of < 45.

The main reason for this divergence involves the characteristics of the service evaluated. The hospital where this research was conducted reopened its hemodynamics service throughout 2022, with full operation starting only in June. Since then, it has become the local tertiary referral center for ACS. As a result, most outpatients were newly diagnosed with CAD following an ACS event, attending their first outpatient consultation after hospitalization. Given that many patients were treatment-naïve, the poor LDL control observed may be partly due to significant sampling bias. Several factors contributed to this issue, including the limited availability of Simvastatin in primary health care, difficulties in acquiring Atorvastatin through specific protocols, the low sociocultural level of the population, dietary habits, sedentary lifestyle, and poor medication adherence.

For arterial hypertension, the BP target was achieved in 10% of patients when considering the strict range specified by the Brazilian Guideline. However, this target was met in 59.6% of cases when using the BP threshold of < 130/80 mmHg, which is the reference used by other studies. Thus, SAH control was similar to the SURF<sup>6,7</sup> (39% to 60%) and EUROASPIRE<sup>8,9</sup> (44%) studies. It is also important to note that a significant number of patients had BP readings below the levels recommended by the guidelines. Approximately 38.7% of patients had SBP < 120mmHg, while 22.6% had DBP < 70mmHg, in addition to 2.2% with DBP < 60mmHg. Excessive SAH treatment, with a significant reduction in blood pressure values (mainly DBP), appears to be related to lower coronary perfusion, which can precipitate coronary events in patients with CAD.18

Regarding diabetes control, HbA1c was within target for 50.8% of diabetic patients, compared to 39-56% in the SURF<sup>6,7</sup> studies and 34.7% in EUROASPIRE<sup>8,9</sup> (the latter, however, considered a strict target of HbA1c  $\leq$  6.5).

With regard to the treatment of CAD and its risk factors, adequate prescriptions were found for most patients, with better percentages when compared to other studies, which again raises the hypothesis of the probable low medication adherence of the sample. While 93.9% of patients used antiplatelets and 100% used statins, the SURF<sup>6,7</sup> and EUROASPIRE<sup>8,9</sup> revealed they found rates between 80.8% and 95.1% of antiplatelet use and between 62% and 85% for statin use.

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The use of ACE inhibitors or ARBs was found in 93.5% of our sample, compared to 47% to 78% in international studies.<sup>6-9</sup> Beta-blockers were prescribed to 93.5% of the patients evaluated, while SURF<sup>6,7</sup> and EUROASPIRE<sup>8,9</sup> reported a prescription of this drug class for 47% to 82.5% of participants. Most patients with diabetes were receiving treatment with oral hypoglycemic agents and/or insulin, and only 7 diabetic patients (7.1%) were not receiving treatment.

In addition to the poor control of risk factors, there were numerous failures in recording data relevant to the treatment of CAD and associated comorbidities. The medical records failed to indicate a history of smoking in 10% of cases, the level of physical activity and data for calculating BMI in approximately 70% of the records. Furthermore, a quarter of the patients did not have up-todate laboratory tests due to a lack of a lipid profile, and a third of the diabetic patients did not have recent HbA1c monitoring.

Therefore, the numerous failures in recording and collecting laboratory tests are major limitations of the work, which can be explained by the lack of adequate guidance, medical requisition at hospital discharge, patients' understanding, as well as scheduling errors by the administrative team. This potentially led to several prevalences being underestimated, including smoking, obesity, frequency and intensity of physical activity. The assessment of dyslipidemia and diabetes management targets may not fully reflect the actual situation. It is worth noting that SURF7 also indicated an approximately 10% overall failure rate of recordings.

In addition to recording errors, other limitations include the lack of standardization in laboratory tests and consultation data collection, such as blood pressure measurements. Isolated BP measurements during consultations can lead to inaccurate treatment prescriptions due to factors such as missed medication on the day of the visit or white coat hypertension. Moreover, the study population may not be highly representative, as it consists primarily of patients from a tertiary hospital, most of whom were discharged after an ACS.

# Conclusion

The model developed in this study is user-friendly and has enabled the evaluation of risk factor prevalence as well as the self-assessment of data recorded in medical records. This approach has provided a comprehensive mapping of the patients attending the Cardiology outpatient clinics at our hospital, facilitating the development of targeted solutions and strategies tailored to this population.

However, the study identified numerous recording errors, underscoring the need for a standardized approach to medical record documentation. This would improve record accuracy and, thus, better manage and control risk factors.

The observed poor control of risk factors highlights the necessity for effective interventions. Since CAD is largely preventable with effective risk factor management, it is crucial to focus on lifestyle changes, medication adherence, and achieving treatment goals.

#### **Author Contributions**

Conception and design of the research: Pacini AF, Bredt Junior GL; acquisition of data and writing of the

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#### **Potential Conflict of Interest**

No potential conflict of interest relevant to this article was reported.

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#### **Ethics Approval and Consent to Participate**

This study was approved by the Ethics Committee of the Universidade Estadual do Oeste do Paraná under the protocol number 61589422.4.0000.0107. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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