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Mean platelet volume (MPV) and incident myocardial infarction and ischemic stroke: A Literature Review

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PREFACE

The aim of this master thesis is to perform a literature review on studies that have assessed the association between mean platelet volume (MPV) and incident arterial cardiovascular disease (i.e., myocardial infarction and ischemic stroke) in order to improve knowledge on the topic.

My interest in thrombosis and hemostasis started in 2019 as I started the integrated research program as a part of medical studies and got accepted to join the Thrombosis Research Group (TREC) at the Institute for Clinical Medicine at University of Tromsø. I had my full year of research in 2021, during COVID-restrictions, but still my supervisors and the rest of the research group made me feel warmly welcomed. I found epidemiology and statistics difficult in the first years of my studies. However, being accepted into a research group of highly qualified, academically inspiring, and including people, my interest in the field of research was quickly triggered. In addition, I early on felt a sense of accomplishment. My work has so far led to one publication as first author: "*Joint Effect of Multiple Prothrombotic Genotypes and Mean Platelet Volume on the Risk of Incident Venous Thromboembolism"*.

Writing this thesis has taught me a lot about research, how to conduct a literature review and how to evaluate the quality of different articles. For the rest of my career as a medical doctor, I will make use of the skills that I now have learned regarding evaluating the quality of research and how to keep me updated on the field of thrombosis and haemostasis.

I would like to give my greatest gratitude to my supervisor Dr. Vânia Maris Morelli for her profound feedback and kind words, and for always being available for questions and discussions regarding this thesis. I would also like to thank Professor Sigrid Kufaas Brækkan and Professor John-Bjarne Hansen for their willingness to share their expertise and always provide feedback. I feel very fortunate and privileged to be a part of the TREC research group.

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Summary

Background: Myocardial infarction (MI) and ischemic stroke (IS) are leading causes of death worldwide. Traditional risk factors, including hypertension, dyslipidemia, smoking and diabetes, account for the majority of the MI and IS cases. However, the disease burden remains substantial, and research for discovering novel risk factors has been ongoing, and large platelets can be a potential candidate. Large platelets, as reflected by an increased mean platelet volume (MPV), has a greater prothrombotic potential compared with small platelets. MPV serves as a marker for platelet function and has been extensively investigated as a prognostic biomarker for people with MI or IS. However, whether MPV is a risk factor for incident MI and IS remains uncertain.

Aim: To perform a review of the existing literature on the potential role of MPV as a risk factor for incident MI and IS.

Methods: A structured literature search was conducted in Medline (February 2023) to identify studies involving an adult population and with a prospective design on the association of MPV with MI or IS. The identified articles were screened with predefined inclusion criteria by title and abstract, or by full-text assessment.

Results: Of 569 identified records, 4 prospective cohort studies were included. Two studies found an association between MPV and the outcomes of interest, where an MPV ≥7.4 fL vs. MPV <7.4 fL (reference) yielded a HR for incident MI of 1.38 (95% CI 1.08-1.75) and an MPV <7.3 fL vs. MPV 7.3-10.3 fL (reference) yielded a HR for incident IS of 0.79 (95% CI 0.65-0.97). The two last studies did not find an association between MPV and the studied outcome. The four included studies had an overall moderate to high risk of bias.

Conclusion: This literature review shows that the available data on the topic is still scarce, and more studies are warranted to establish the role of platelet size, as reflected by MPV, for the risk of incident MI or IS and in the pathophysiology of both diseases.

Abbreviations

IS – Ischemic stroke

MI – Myocardial infarction

BMI – Body mass index

CHD – Coronary heart disease

CI – Confidence interval

HDL – High density lipoprotein

HR – Hazard ratio

LDL – Low density lipoprotein

OR – Odds ratio

RR – Relative risk

TIA – Transient ischemic attack

WHO – World Health Organization

1. Introduction

Myocardial infarction (MI) can be defined as a damage to the myocardium (the heart muscle) due to the lack of oxygen delivery as a result of partially or fully obstructed blood flow in the coronary arteries [1]. Together with MI, stroke is one of the most severe forms of arterial cardiovascular disease (CVD). Stroke is defined by World Health Organization (WHO) as a clinical syndrome consisting of rapidly developing clinical signs of focal (or global in case of coma) disturbance of cerebral function lasting more than 24 hours or leading to death with no apparent cause other than a vascular origin [2]. Ischemic stroke (IS) is the most common presentation of stroke in Europe, accounting for about 87% of the strokes, with the remaining 13% being hemorrhagic strokes [3, 4]. The traditional major risk factors for MI and IS are hypertension, dyslipidemia, smoking and diabetes [5-7]. Even though these risk factors account for the majority of MI and IS cases occurring in the general population [6, 7], intensive research has been carried out over the last decades to identify novel risk factors involved in the pathophysiology of both diseases. One of these potential risk factors are large platelets. Platelets differ in size, as reflected by their mean platelet volume (MPV), which is a measure of the average size of circulating platelets and a marker of platelet reactivity [8]. Large platelets, as reflected by an increased MPV, compared to small platelets are more metabolically and enzymatically active and prone to express phosphatidylserine on their membrane and could therefore have a greater prothrombotic potential [8, 9]. It is worth noting that MPV is a particularly interesting candidate biomarker for the assessment of MI or IS risk, because it is a trait with a strong genetic component [10, 11] and known to be relatively stable within an individual over time, especially in healthy subjects [12, 13]. MPV has been investigated mainly as a prognostic biomarker for adverse outcomes, such as mortality, in people with MI and IS, but whether MPV is a risk factor for incident MI and IS remains uncertain.

The aim of this thesis is to perform a review of the existing literature on the potential role of MPV as a risk factor for incident MI and IS. Results from this literature review may direct further research efforts aimed to improve knowledge on the association of MPV with incident MI or IS and the role of platelet size in the pathophysiology of both diseases.

2. Myocardial infarction and ischemic stroke

2.1 Epidemiology

Cardiovascular diseases (CVDs) are highly prevalent worldwide and the leading cause of death [14]. Among CVDs, MI was the top cause of death with 19.1 million deaths in 2022 [14], accounting for 32% of all deaths globally [15], which is more than twice as much as deaths caused by cancer [16]. Acute MI is the most severe form of coronary artery disease (CAD) (Figure 1) and is subdivided into "ST-segment elevation myocardial infarction" (STEMI) where one or more of the coronary arteries are fully blocked, or "non-ST-segment elevation myocardial infarction" (NSTEMI), where the coronary arteries are partially occluded [17]. CAD can also be named coronary heart disease (CHD) or ischemic heart disease (IHD) [18]. According to the American Heart Association, it was estimated that in 2020, 244.1 million people were living with CHD globally, and it was more prevalent in males than in females (141.0 and 103.1 million people, respectively) [14].

The incidence of MI has significantly decreased since the late 1900s [19]. In part, the reduction seems to be driven by the decrease in the incidence of STEMI from 47% (1999) to 22.9% (2018) [19]. The reduction in STEMI is suggested to be due to considerable improvements in primary-prevention efforts by public health targeting the well-established modifiable cardiometabolic risk factors, such as hypertension, dyslipidemia, diabetes mellitus, smoking and abdominal obesity [6, 20]. However, despite the decrease in mortality and the substantial improvements in prevention and prognosis over the past decades, more than 4 million people will die from CVDs each year across Europe, and MI still remains the leading cause of death worldwide [16, 21].

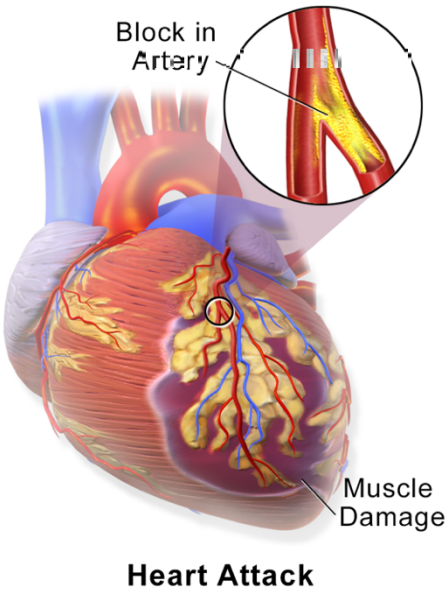

Figure 1. Illustration of myocardial infarction (MI). Macroscopic view of the heart with a thrombus forming in the left anterior ascending coronary artery (LAD). The resulting cessation of blood flow leads to ischemia and necrosis in the part of the myocardium. MI can be both STEMI or NSTEMI, depending on the degree of occlusion in the artery. This figure shows a STEMI with a fully occluded artery in LAD. STEMI, ST-segment elevation myocardial infarction; NSTEMI, non-ST-segment elevation myocardial infarction. *By Blausen Medical Communications, Inc., CC BY 3.0 https://creativecommons.org/licenses/by/3.0, via Wikimedia Commons*

Stroke is the second-leading cause of death and disability in the world [7], with around 1.1 million inhabitants in Europe suffering a stroke every year. Stroke is defined by the World Health Organization (WHO) as a clinical syndrome consisting of rapidly developing clinical signs of focal (or global in case of coma) disturbance of cerebral function lasting more than 24 hours or leading to death with no apparent cause other than a vascular origin. Of all stroke types, about 87% are ischemic and 13% hemorrhagic [22]. Among the IS, approximately 20-30% are cardioembolic [23], with an embolus originating from the heart or aorta, and the remaining originating from local thrombi formed within the cerebral arteries [24].

2.2 Pathophysiology

The pathophysiology behind arterial thrombosis (both MI and IS) is mainly triggered by the formation and subsequent rupture of an atherosclerotic plaque. In the lining of an arterial vessel wall the development of atherosclerosis is initiated by the buildup of an atherosclerotic plaque, a process that often occurs over several years [25]. The vessel lumen is shrinking during the growth of the plaque due to deposition of substances such as lipids in the core of the plaque. The lipid core has highly thrombogenic properties and is also known as the necrotic core. For instance, MI often occurs as a result of the rupture or erosion of an atherosclerotic plaque, leading to exposure of its thrombogenic core to the circulation. Thereafter, the hemostatic system is stimulated and a platelet plug is formed in the inner lining of a vessel wall, growing to become a platelet-rich thrombus (Figure 2). As the thrombus increases in size, it can occlude the vessel lumen and result in comprised tissue perfusion to the myocardium, eventually leading to ischemia and necrosis [26]. Although the formation of an atherosclerotic plaque within cerebral arteries is a well-established mechanism for IS, 20-30% [23] of the cases can be cardioembolic. In this case, the thrombus is generally formed in the atrium as a consequence of atrial fibrillation (AF).

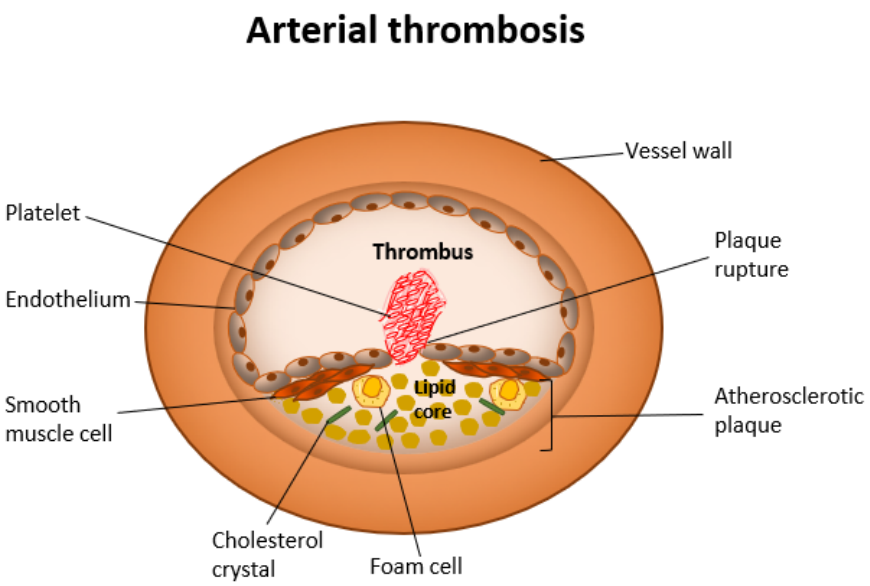

Figure 2. The primary trigger of arterial thrombosis is rupture of an atherosclerotic plaque. This involves disruption of the endothelium and release of constituents of the plaque into the lumen of the blood vessel.

2.3 Risk factors

MI and IS share several common well-established traditional risk factors, such as hypertension, dyslipidemia, smoking and diabetes [5]. A large study found that 90% of the risk of MI can be attributed to these common modifiable risk factors [6]. Further, around 88% of ischemic stroke disability-adjusted life years (DALYs) may be due to the above-mentioned common risk factors [7]. This demonstrates the tremendous possibility for decreasing the burden of MI and IS through reductions in exposure to these risk factors. Indeed, extensive research on modifiable risk factors over the last decades has resulted in successful implementation of preventive measures and targeted intervention (e.g., statins to lower cholesterol levels or blood pressure lowering agents), leading to a declining incidence of MI and IS [27, 28]. However, such decline has taken place mainly in high-income countries. The majority of the global disease burden is now in low- and middle-income countries, with an age-standardized stroke-related mortality rate that is 3.6 times higher in low-income countries than in high-income countries [7, 29].

2.3.1 Potential novel risk factors of MI and IS

Despite the fact that the traditional risk factors account for much of the burden of MI and IS [5], there is intensive research focus on discovering novel risk factors for atherosclerosis development, and large platelets are among the candidates. As addressed in the former

section, platelets play an important role in the pathophysiology of MI and IS. Platelets differ in size, as reflected by the MPV, which is a measure of the average size of circulating platelets and a marker of platelet reactivity [8]. The notion that large platelets represent a more reactive subpopulation is supported by *in vitro* studies, where large platelets compared to small platelets were found to adhere faster and exhibit stronger aggregation after stimulation with common agonists, such as adenosine diphosphate (ADP), collagen or thrombin [30-32]. Other functional differences, such as increased granule release and integrin activation, were also seen in large platelets in comparison with the small ones [33]. Further, large platelets are more prone to expose negatively charged phospholipids (i.e., phosphatidylserine) on their membranes [8]. Taken together, these biological properties could lead to an increased prothrombotic potential and be a mechanism by which large platelets, as reflected by an increased MPV, would increase the risk of CVDs. Platelet traits are highly heritable [10, 11, 34], and studies have shown that MPV is relatively stable in healthy subjects over time [12, 13], making it well suited as a potential biomarker for assessing CVD risk. Moreover, an elevated MPV is associated with the risk factors for arterial CVD, such as diabetes mellitus [35], hypertension [36], hypercholesterolemia [37], smoking [38], obesity [39]. Considerable research has been carried out on MPV in relation to arterial CVD as a diagnostic and mainly prognostic biomarker for adverse outcomes among people with MI or IS, as summarized in the next section.

MPV as a diagnostic and prognostic biomarker for MI and IS

Previous studies have mainly focused on MPV as a prognostic biomarker for adverse events, such as all-cause mortality. Two systematic reviews, comprising 41 studies [40] and 16 studies [41], investigated the association between MPV at hospital admission and adverse outcomes after acute coronary syndrome (ACS). In these systematic reviews, an increased MPV was associated with a higher risk of all-cause mortality after acute MI [41] and long-term risk of mortality [40]. MPV has also been studied in relation to restenosis after percutaneous coronary intervention, showing that baseline mean MPV was significantly higher in patients who developed restenosis than those who did not [41]. Moreover, elevated MPV following an MI has been reported to be an independent risk factor for recurrent ischemia [42, 43]. With regards to cerebrovascular events, a meta-analysis comprising 34 articles using case-control or cross-sectional designs found that MPV was significantly higher in patients with acute stroke compared to controls [44]. Several studies have also reported

associations of MPV with stroke severity [45, 46], death or hospital readmission [47], and poor outcome after mechanical thrombectomy [48].

Some studies have also assessed if MPV could be used as a diagnostic biomarker in patients with acute MI. A Taiwanese study with 282 patients investigated the ability of MPV to detect acute ACS in patients admitted to the emergency department because of chest pain. Results from the study showed that MPV levels were significantly higher among patients with acute MI than among those without MI, and demonstrated the possible ability of MPV to be predictive of ACS in an acute phase [49]. These findings are supported by other studies from Taiwan [50] and Iran [51], however, all of the studies have small sample size and limited statistical power.

2.4 Rational to perform a literature review on MPV as a biomarker for incident MI and IS

MPV is the measure of average size of circulating platelets that reflects platelet function, and a high MPV is an indirect marker of increased platelet reactivity [8]. Platelets secrete and expose several substances that mediate key pathophysiological processes, including inflammation, thrombosis, and atherosclerosis [52]. Therefore, it is biologically plausible that large platelets, as reflected by an increased MPV, might have a role in the development of arterial thrombosis. As previously mentioned, there is a considerable number of studies assessing MPV as a predictive biomarker for adverse outcomes after MI and IS. However, whether MPV is associated with risk of a first-lifetime MI or IS remains uncertain. A comprehensive literature review on the association between MPV and the risk of incident MI or IS, including the most recent epidemiological studies, is an important step to provide an updated overview and address the most important knowledge gaps related to the topic, directing further research efforts.

Aim of the thesis

The aim of this thesis is to perform a review of the existing literature on the potential role of MPV as a risk factor for incident MI and IS.

3. Methods

A structured literature search was conducted to identify relevant studies regarding the association of MPV with incident IS or MI. The paper on "Conducting Systematic Reviews and Meta-Analyses of Observational Studies of Etiology (COSMOS-E)" [53] was used as guidance, where the concept of PECO is presented. In line with the well-known Population, Intervention, Control, and Outcome (PICO) format for intervention studies, the concept of Population, Exposure, Control, and Outcome (PECO) has been developed as a framework for formulating good research questions to explore associations in systematic reviews of observational studies [54].

3.1 Data source and search strategy

A literature search in Medline was carried out to obtain articles of interest and was conducted in February 2023 by searching through a combination of all relevant MESH-terms and free text words related to MI (e.g., myocardial infarction, coronary artery disease, ischemic heart disease, ischemic cardiac disease, and acute coronary syndrome), IS (e.g., stroke, brain infarction, cerebral stroke, and cerebral insult) and MPV (e.g. mean platelet volume, mean thrombocyte volume and average thrombocyte volume). The search was restricted to studies involving humans, and due to limited resources, only articles in English language were included. An overview of the literature search is described in Figure 3, and details on the search strategies are presented in Supplementary Table 1. After the search was performed in Medline, the articles were imported to EndNote XP (Thomson Reuters, Toronto, Canada) and thereafter to Rayyan [55] to manage and organize the literature and perform the screening. In order to ensure that the search was comprehensive, the electronic search was supplemented by a manual search by carefully reading the reference list of all systematic reviews found in the original search in Medline, together with the reference list of all included studies.

Fig. 3. Overview of the combination of words in the search strategy.

3.2 Inclusion and exclusion criteria

Studies involving adults (≥ 18 years old) were included regardless of date of conduction and publication, the geographic area or ethnic group. The literature search was limited to one exposure, i.e., MPV, and the outcomes were MI or IS that were objectively confirmed or assessed by International Codes of Disease (ICD-codes) [56]. STEMI and NSTEMI are frequently evaluated together in epidemiological research as their therapy is often similar, therefore articles on MI regardless of being STEMI or NSTEMI were included [17, 21, 57]. Finally, CAD, CHD or IHD were included as outcomes since these terms also comprise MI.

Cohort studies (either prospective or retrospective), case-cohort studies and nested case-control studies were included in order to establish a clear temporal sequence between exposure and the outcome through this type of study design. Consequentially, case-control and cross-sectional studies were excluded as the temporal relationship between exposure and outcome cannot be determined. In addition, case-reports, case-series and reviews (including systematic reviews) were excluded. Studies on MPV as a prognostic factor for adverse outcomes of those with MI and IS, and studies on MPV as a diagnostic biomarker for those with acute events were excluded.

Titles and abstracts of all retrieved records were initially screened, and thereafter, full-text assessment was performed according to the above-mentioned predefined inclusion and exclusion criteria.

3.3 Extraction and analysis of data

In order to present the included studies coherently, the following variables were extracted and presented in a table: first author, year of publication, country, study design, number of study participants, length of follow-up, median/mean age and proportion of men, outcomes of interest, main results, and conclusions. For this master thesis, only a qualitative synthesis of the included studies was performed.

3.4 Assessing the risk of bias in the individual studies

The risk of bias was assessed in each study by using a summarized version of the Quality in Prognosis Studies tool, which consists of six bias domains to be considered: study participation, study attrition, exposure measurement, outcome measurement, confounding measurement and adjustments, and statistical analysis and reporting [58].

4. Results

The literature search in Medline identified 569 records (see Figure 4 for PRISMA flowchart). The articles were screened by title and abstract, and 548 were excluded as most of them focused on patients with pre-existing CVDs and searched for MPV as a predictive biomarker for adverse outcomes. After exclusion, 21 articles were identified as relevant and underwent full text assessment. Among these articles, 17 were excluded as they were commentaries, editorials, case-control studies or their full text were not retrievable in PubMed or other databases. To ensure the completeness of the literature search, a manual review of 9 relevant systematic reviews was conducted for this purpose. Reference lists of the systematic reviews were examined to search for eligible articles. Most of the articles were excluded due to study design (mainly case-control studies) or because they only assessed MPV as a prognostic factor in people with arterial CVD. The full reference lists of the included articles in this review were also revised to search for eligible articles. No further articles met the inclusion criteria and were therefore not included. As described in the flowchart (Figure 4), four studies were included for qualitative data analyses, and the next section is dedicated to present these studies.

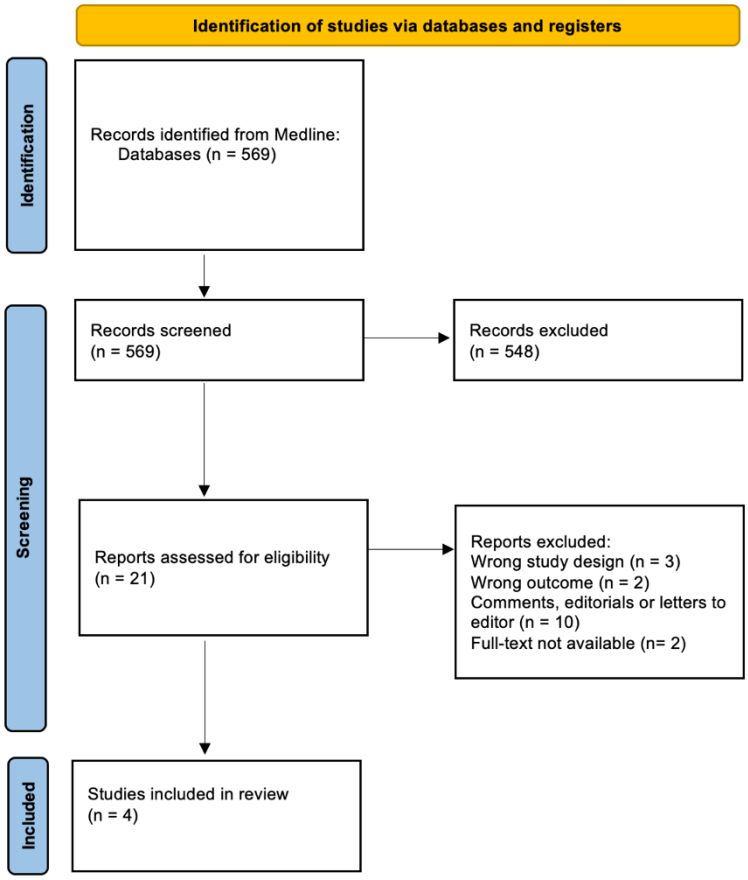

Figure 4. Flow chart of the literature review according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [59].

4.1 Description of included studies

The description of the included studies is summarized in Table 2. In a prospective cohort study derived from the Copenhagen General Population Study (Denmark), Klovaite et al [60] assessed the association of MPV with incident MI and all-cause mortality. Around 39,000 participants without previous history of MI were followed from blood sampling until the occurrence of MI, death or May 2009 (maximum of 6 years of follow-up). The identification of MI was carried out searching the national Danish Patient Registry and the national Danish Causes of Death Registry, with the use of ICD-codes. The study found that an increased MPV was associated with a higher risk of incident MI. Participants with MPV ≥7.4 fL compared to those with MPV <7.4 fL had a 1.4-fold (HR 1.38, 95% CI 1.08-1.75) increased risk of incident MI in analysis adjusted for age, sex, dyslipidemia, high-sensitivity C-reactive protein, body mass index (BMI), comorbidities (hypertension and diabetes mellitus), smoking, and time from blood sampling until laboratory analysis of MPV [60].

In a study conducted by He et al. [61], authors conceived a prospective cohort composed of retired employees from the Dongfeng Motor Corporation (DMC) in China, with recruitment dating from 2008 to 2013. For the MPV analysis, the cohort included 25,591 participants without previous history of CHD and stroke. The identification of stroke and CHD, which also included MI, was carried out by reviewing the medical insurance systems and medical records in the DMC-owned hospital, with the use of ICD-codes. During a median follow-up of 5.9 years, there were 3,738 incident CHD events and 1,059 incident stroke events, of which 840 were IS [61]. The study revealed a protective effect of a low MPV on the risk of CHD and stroke, as decreasing levels of MPV were significantly associated with a reduced risk for the studied outcomes [61]. Compared to the middle category (MPV: 7.3-10.3 fL), those in the lowest category of MPV (<7.3 fL) had HRs of 0.80 (95% CI 0.73-0.88) for CHD, 0.84 (95% CI 0.71-1.00) for stroke (combined ischemic and hemorrhagic) and 0.79 (95% CI 0.65-0.97) for IS only, in analyses adjusted for age, sex, lifestyle factors, education, BMI, comorbidities (hypertension, dyslipidemia, diabetes), anti-platelet agents, and family history of CVD [61].

In contrast to the Danish and Chinese studies [60, 61], the European Prospective Investigation into Cancer and Nutrition-Netherlands Study (EPIC-NL) [62] and the Malmö Diet and Cancer Study [63] found no association between MPV and arterial CVD, MI or stroke. The EPIC-NL was a prospective population-based cohort study composed of 14,362 adults free of CVD at baseline (enrollment from 1993-1997). The identification of the

outcomes of interest (i.e., stroke and CHD, which also included MI) was carried out searching the National Medical Registry from hospital discharge diagnosis database, with the use of ICD-codes. After a median follow-up of 11.4 years, 589 participants developed incident CHD and 196 developed incident stroke (with no differentiation between ischemic and hemorrhagic). For the analyses, MPV was categorized according to clinically relevant cut-off values. When comparing the lowest (<7.5 fL) or highest (≥11.5 fL) MPV categories to the middle category of MPV (7.5-11.5 fL), no association was observed for CHD and stroke, in analyses adjusted for age, sex, lifestyle factors (physical activity, smoking and alcohol intake), education, BMI, waist-to-hip ratio, and comorbidities (hypertension, dyslipidemia, diabetes) [62].

Finally, the Malmö Diet and Cancer Study is a prospective population-based cohort study, encompassing 30,314 participants recruited between 1991 and 1996, of whom 601 had prevalent MI and 344 had prevalent stroke [63]. The identification of new cases of MI and IS during follow up was carried out by linking a unique 10-digit personal identification number with the Swedish National Hospital Discharge Register (SNHDR) and Stroke Register of Malmö (STROMA), with the use of ICD-codes. Participants were followed until 2014, with a median follow-up of 16.1 years. The number of participants developing adverse events during follow-up were: 6,110 individuals with all-cause death, 2,051 with cardiovascular death, 2,482 with MI, and 2,300 with IS. Authors found no association of MPV with MI, with a HR of 1.04 (95% CI 0.89-1.20) when comparing the highest (>9.5 fL) versus lowest (≤8.4 fL) MPV quintile in analyses adjusted for age, sex, BMI, comorbidities (diabetes, hypertension, congestive heart failure, atrial fibrillation, cancer, previous stroke, previous MI), and drug therapy (e.g., antiplatelet and antidiabetic drug). Although the 95% CIs included the unit, an association between elevated MPV and IS seemed to be present, with a HR of 1.17 (95% CI 0.99-1.37) for the highest versus lowest quintile [63].

4.2 Bias assessment

All included studies had either moderate (n= 2) or high (n= 2) overall risk of bias. Summary assessment of the risk of bias is shown in Table 2. The description of **study participation** was clear in most of the studies [61-63], but the study conducted by Klovaite et al. [60] did not mention the precise number of participants eligible for follow-up in the time-to-event analysis. The risk of bias in relation to **study attrition** was high or moderate in all included studies, mainly due to missing reporting on number and reasons for loss-to-follow-up, with no description of potential differences between participants who completed the study and those

who did not. Risk of bias in relation to **prognostic factor measurements** was moderate [60, 61, 63] or low [62]. Of note, most of the studies did not report whether there were pre-analytical or analytical methodological differences in the laboratory measurement of MPV among study participants (e.g., time between blood sampling and measurement of MPV, and the use of different cell counters). The risk of bias due to **outcome measurements** was considered moderate or high. All the studies used ICD-codes for the diagnosis of the outcomes, which can potentially lead to some degree of misclassification, and one study did not report the number of participants developing the outcome of interest [60]. Further, the study conducted by Patti et al. [63] did not report whether the participants who developed MI or IS during follow-up had previous history of these diseases. All studies accounted for the traditional risk factors for arterial CVD as potential confounders, yielding a moderate to low risk of bias in the domain related to **confounding**. The studies in general were not clear regarding the methods (e.g., laboratory measurement) to assess the variables considered potential confounding. The description of the **statistical analysis and reporting** of results was unclear in two studies [60, 63] due to insufficient presentation of data to assess the adequacy of the analytic strategy, yielding a moderate risk of bias.

Table 1 Characteristics of the included studies

First Author/ (Year of publication)	Country	Study design	Number of participants	Follow-up period	Age at cohort inclusion and % of men	Outcome	Main findings	Conclusion
Klovaite *et al.* (2011) [60]	Denmark	Prospective cohort study "Copenhagen General Population Study"	~39,000	Up to 6 years	Mean: 57 years, and 46% men	MI	MPV ≥7.4 fL vs MPV <7.4 fL (reference): **HR for MI of 1.38 (95% CI 1.08-1.75)** in multivariable analysis	Increased MPV is associated with increased risk of MI independent of known risk factors of MI
He *et al.* (2019) [61]	China	Prospective cohort study "The Dongfeng-Tongji Cohort Study"	25,591	Median: 5.9 years	Age and % of men according to MPV categories [a]	CHD (including MI), all strokes (ischemic and hemorrhagic) and IS only	MPV <7.3 fL vs. MPV 7.3-10.3 fL (reference): **HRs of 0.80 (95% CI 0.73-0.88) for CHD, 0.84 (95% CI 0.71-1.00) for stroke, 0.79 (95% CI 0.65-0.97) for IS** in multivariable analysis	The study revealed a protective effect of a low MPV on the risk of CHD and IS
Lassale *et al.* (2018) [62]	The Netherlands	Prospective cohort study "EPIC-NL"	14,362	Median: 11.4 years	Mean: 47.8 ±11.7 and 28.3% men	CHD (including MI) and stroke (both ischemic and hemorrhagic)	MPV ≥11.5 fL vs MPV 7.5-11.5 fL (reference): **HRs of 1.18 (95% CI 0.74-1.90) for CHD and 0.72 (95% CI 0.27-1.94) for stroke** in multivariable analysis	Elevated MPV is not associated with increased risk of CHD and stroke
Patti *et al.* (2019) [63]	Sweden	Prospective cohort study "Malmö Diet and Cancer Study"	30,314 (601 with prevalent MI and 344 with prevalent stroke)	Median: 16.1 years	57 ± 8 and 40% men	All-cause death, cardiovascular mortality, MI and IS	MPV >9.5 fL vs MPV ≤8.4 fL (reference): **HRs of 1.04 (95% CI 0.89-1.20) for MI and 1.17 (95% CI 0.99-1.37) for IS** in multivariable analysis	Elevated MPV is not associated with increased risk of MI but there might be an association with IS

[a] Mean age and proportion of men in different MPV categories: <7.3 fL (61.9±7.9; 45.7%), 7.3-10.3 fL (61.8±8.0; 44%), >10.3 fL (60.8±8.3; 40.5%).

Abbreviations: CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; EPIC-NL, European Prospective Investigation into Cancer and Nutrition-Netherlands Study; HR, hazard ratio; IS, ischemic stroke; MI, myocardial infarction; MPV, mean platelet volume.

Table 2. Rating the risk of bias according to Quality in Prognosis Studies tool [58]

First Author	Study participation	Study Attrition	Prognostic Factor Measurements	Outcome measurements	Study confounding	Statistical Analysis and Reporting	Overall risk of bias
Klovaite *et al.* (2011) **[60]**	High	High	Moderate	High	Moderate	Moderate	High
He *et al.* (2019) **[61]**	Low	Moderate	Moderate	Moderate	Low	Low	Moderate
Lassale *et al.* (2018) **[62]**	Low	Moderate	Low	Moderate	Moderate	Low	Moderate
Patti *et al.* (2019) **[63]**	Low	High	Moderate	High	Moderate	Moderate	High

5. Discussion

5.1 General discussion

This is the first literature review, to the best of our knowledge, aimed to evaluate the association between MPV and the risk of incident MI or IS. Among the 569 records retrieved from Medline, 4 large prospective cohort studies meeting the inclusion criteria were identified. Among the included studies [60-63], two found an association between MPV and the outcomes of interest [60, 61]. The results of these studies were somehow consistent, since one study found an association between increased MPV and increased risk of MI [60], and the other found that decreased levels of MPV were related to lower risk of CHD, stroke, and, particularly IS [61].

The analysis of the literature review is based on 4 studies, including approximately 100,000 subjects [60-63]. Three of the included studies are population based with participants recruited from the general population. The blood samples to measure MPV were collected at baseline, and the participants were followed over several years. Due to the prospective design, the included studies were able to establish a temporal relationship between the exposure (MPV) and the outcomes of interest (MI or IS). The included studies have also assessed and accounted for several cardiometabolic factors (e.g., obesity, hypertension, diabetes, and dyslipidemia) related to both MPV and arterial CVD that could act as potential confounders. Nonetheless, the findings of the 4 studies were not consistent. For instance, in the Danish study conducted by Klovaite et al. [60] and in the Chinese study conducted by He et al. [61], MPV was associated with the risk of incident MI, CHD and IS. However, a study carried out in the Netherlands [62] found no association of MPV with CHD and stroke. It is important to address that the Dutch study did not differentiate between ischemic and hemorrhagic stroke, and the population was relatively young (~48 years at baseline) and composed mainly of women (~70%). All these factors might have contributed to the null findings of the aforementioned study. Further, the Swedish study conducted by Patti et al. [63] found no association between MPV and MI. It is worth noting that in this study an association between elevated MPV and IS appeared to be present, as the HR for the highest versus lowest MPV quintile was 1.17 (95% CI 0.99-1.37), however the 95% CI included unity. A concern that merits attention is the fact that the Swedish study included prevalent cases and did not perform a separate analysis excluding these prevalent cases [63]. This concern will be further addressed in the section of methodological discussion.

The mechanisms of the potential association of MPV with risk of incident MI or IS remains to be elucidated. One of the most convincing evidence of platelets as a key component in arterial thrombosis comes from trials of antiplatelet drugs, and studies on platelet activation in acute ischemic syndromes [64]. The consistent findings of 50% reduced risk of MI or death in patients with unstable angina taking aspirin supports the notion that platelets are crucial for the development of MI [65]. Moreover, trials of platelet inhibitors [66] and studies of biochemical measurements [67] have shown that platelet activation plays an important role in IS. From a biological viewpoint, it is reasonable to assume that large and hemostatically more active platelets, as reflected by an increased MPV, might influence the pathogenesis of MI or IS [68], as increased platelet activation represents a risk factor for atherothrombosis [69, 70]. Several acquired conditions may influence MPV, including obesity [39], hypertension, diabetes, and smoking [71]. Further, heritability studies in twin- and family-based cohorts have demonstrated that genetic factors are important determinants of platelet count and MPV [10, 11]. Some of the findings of this literature review [60, 61] suggest that platelet size, as reflected by MPV, might be involved in the development of incident events of MI or IS, even after extensive adjustment for several cardiovascular risk factors.

5.2 Methodological discussion

The main strength of this thesis is the structured and comprehensive literature search with predefined inclusion criteria in Medline, a major database for medical research. A thorough literature search combined with crosschecking of references by carefully reading the reference list of included articles and systematic reviews, enhances the possibility of a comprehensive overview of the available literature on the topic. However, it also important to underscore that the literature search was not conducted systematically and other databases, such as Embase, were not included. Therefore, the possibility that not all relevant articles were identified cannot be ruled out. Further, the literature search and screening were performed by only one person, and the possibility that some papers of interest might have not been identified during the screening process cannot be excluded.

Prospective cohort studies avoid the issue of reverse causation, as there is a clear temporal sequence between the exposure and outcome in this design. However, cohort studies, as all observational studies, are susceptible to confounding [72]. Although extensive adjustment for cardiometabolic risk factors was conducted in all included studies [60-63],

residual confounding cannot be excluded. Although MPV has been shown to be a relatively stable phenotype within an individual over time [12, 13], MPV values could have changed during the long-term follow-up of the cohort studies [60-62], introducing the possibility of regression dilution, [73], and a weakening of the true potential association of MPV with MI or IS. Regarding the selection of the study population, in the Swedish study conducted by Patti et al [63], 2% and 1% of the included participants had prior history of MI and stroke, respectively. Although the percentage of prevalent cases was low, the risk of developing the studied outcome might have differed from those who were "free" of the disease at baseline, introducing the possibility of a distortion in the relationship between the exposure and outcome. Finally, all included studies [60-63] did not clearly report the numbers and reasons for loss to follow-up in the time-to-event analysis. If participants who are lost to follow-up and those who remain in the cohort have different probabilities of developing the studied outcome, a selection bias may be introduced.

Another limitation of all included studies is the fact that they used ICD-codes to identify the studied outcome, which might have led to some degree of misclassification. Further, studies did not use the same ICD-codes to identify MI, even though all of them used the I.21-I.22 codes (I.21: "Acute MI" and I.22: "Subsequent STEMI and NSTEMI"). In addition, the Swedish [63] and Dutch [62] studies used broader ICD-codes that included angina pectoris, acute MI, STEMI and NSTEMI. As previously mentioned, the Dutch study did not use any strategy to differentiate between ischemic and hemorrhagic stroke [62].

Other possible limitations are the uncertainties around a clinical normal range for MPV. Indeed, all the included studies used different cut-off values to categorize MPV. In addition, there are concerns related to blood collection and analysis of MPV, such as the type of hematology analyzer and the time between blood sampling and the measurement of MPV. Pre-analytical conditions of blood samples, such as type of anticoagulant, might impact the values of blood cells, including platelets. EDTA requires a low time-to-analyze as it is shown to impact the platelet size through swelling in a time-dependent manner [74]. Among the included studies, only Klovaite et al. adjusted for time from blood sampling to measurement of MPV in their multivariable analyses [60]. However, if the time between blood sampling and measurement of MPV was not related to the outcome in the other studies [61-63], potential measurement errors would have led to non-differential misclassification, thereby introducing a possibility for underestimation of the true associations.

In conclusion, this literature review shows that the association of MPV with incident MI and IS remains unclear. As data on the topic is still scarce, future studies are needed to establish the role of platelet size, as reflected by MPV, in the risk of incident MI or IS and in the pathophysiology of both diseases.

6. Supplemental material

Supplementary table 1. Search strategy Medline

OVERVIEW		
Interface: Ovid Medline **Database:** Ovid Medline **Date of search:** 21.02.2023 **Limits:** English language, studies on humans		
SEARCH STRATEGY		
Search terms		
Ischemic stroke block:		
1	cerebrovascular disorders/ or exp brain infarction/ or exp hypoxia-ischemia, brain/ or exp stroke, lacunar/ or exp "intracranial embolism and thrombosis"/ or exp ischemic stroke/	MeSH-terms
2	/Brain isch*.ti,kw	Free-text terms
3	/Brain infarction.ti,kw	Free-text terms
4	/Brain hypoxia-ischaemia.ti,kw	Free-text terms
5	/Lacunar stroke.ti,kw	Free-text terms
6	/Intracranial arter* disease*.ti,kw.	Free-text terms
7	/Middle cerebral artery infarction.ti,ab,kw	Free-text terms
8	/Anterior cerebral artery infarction.ti,ab,kw	Free-text terms
9	/Posterior cerebral artery infarction.ti,kw.	Free-text terms
10	/Intracranial Embolism.ti,kw	Free-text terms
11	/Intracranial Thrombosis.ti,kw	Free-text terms
12	/Stroke.ti,kw	Free-text terms
13	/Cerebral stroke.ti,ab,kw	Free-text terms
14	/Cerebr* stroke.ti,kw	Free-text terms
15	/Brain infarction.ti,kw	Free-text terms
16	/Ischemic stroke.ti,ab,kw	Free-text terms
17	/Ischaemic stroke,ti,ab,kw	Free-text terms
18	/Isch* stroke.ti,ab,kw	Free-text terms
19	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18	

Search terms		
Myocardial infarction block:		
20	myocardial ischemia/ or exp acute coronary syndrome/ or exp angina pectoris/ or exp coronary artery disease/ or exp coronary occlusion/ or exp coronary thrombosis/ or exp anterior wall myocardial infarction/ or exp inferior wall myocardial infarction/ or exp non-st elevated myocardial infarction/ or exp st elevation myocardial infarction/	MeSH-terms
21	/Myocardial ischemia.ti,kw	Free-text term
22	/Coronary syndrome.ti,kw	Free-text term
23	/Angina pectoris.ti,kw	Free-text term
24	/Anterior wall myocardial infarction.ti,kw	Free-text term
25	/Posterior wall myocardial infarct*.ti,kw	Free-text term
26	//Inferior wall myocardial infarct*.ti,kw	Free-text term
27	/Non-ST elevated myocardial infarction.ti,kw	Free-text term
28	/ST elevat* myocardial infarction.ti,kw	Free-text term
29	/Anterior wall myocardial infarct.ti,kw	Free-text term
30	/Inferior wall myocardial infarct.ti,kw	Free-text term
31	/Non-ST elevat* myocardial infarct*.ti,kw	Free-text term
32	/Coronary artery disease.ti,kw	Free-text term
33	/Coronary occlusion.ti,kw	Free-text term
34	/Coronary thrombosis.ti,kw	Free-text term
35	/Myocard* isch*.ti,kw	Free-text term
36	/Myocard* infarc*.ti,ab,kw	Free-text term
37	20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36	
Search terms		
Mean platelet volume block:		
38	Mean platelet volume.ti,ab,kw	Free-text term
39	Average trombocyte volume.ti,ab,kw.	Free-text term
40	Mean thrombocyte volume.ti,ab,kw.	Free-text term
41	38 or 39 or 40	

42	19 or 37	
43	41 and 42	
44	limit 42 to (english language and humans)	

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