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Association of Inflammatory Biomarkers with Morbidity and Mortality Risk in Patients with Peripheral Artery Disease: A Systematic Review and Meta-Analysis

Maria Francisca de Vasconcelos e Marques

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FACULDADE DE **MEDICINA**

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Eu, Maria Francisca de Vasconcelos e Marques, abaixo assinado, nº mecanográfico 201906969, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter actuado com absoluta integridade na elaboração do meu trabalho de Dissertação ou Monografia.

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Association of Inflammatory Biomarkers with Morbidity and Mortality Risk in Patients with Peripheral Artery Disease: A Systematic Review and Meta-Analysis

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Faculdade de Medicina da Universidade do Porto, 15/03/2025

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UC Dissertação - DECLARAÇÃO DE TRANSPARÊNCIA RELATIVAMENTE À UTILIZAÇÃO DE FERRAMENTAS DE CHATBOT GENERATIVO BASEADAS EM LARGE LANGUAGE MODELS

Eu, Maria Francisca de Vasconcelos e Marques, abaixo assinado, nº mecanográfico 201906969, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro que:

☐ Não procedi à utilização de ferramentas de *chatbox* generativo baseadas em *large language models* para nenhuma das tarefas no contexto do meu trabalho de Dissertação ou Monografia

☐ Procedi à utilização de ferramentas de *chatbox* generativo baseadas em *large language models* no contexto do meu trabalho de Dissertação ou Monografia, encontrando-se todas as interacções (*prompts* e respostas) transcritas em anexo bem como a indicação das aplicações utilizadas.

Neste sentido, confirmo que a eventual utilização de ferramentas de *chatbox* generativo baseadas em *large language models* no contexto do meu trabalho de Dissertação ou Monografia foi exclusivamente descrita na sequência de *prompts* e respostas transcritos em anexo e nas aplicações indicadas.

Faculdade de Medicina da Universidade do Porto, 15/03/2025

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Francisca Marques

Dedicatória

Agradeço profundamente ao meu orientador, Doutor João Rocha Neves, e à minha co-orientadora, Doutora Mariana Marques, pela orientação dedicada, paciência e pelo incentivo contínuo, que foram essenciais para o meu crescimento académico.

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Association of Inflammatory Biomarkers with Morbidity and Mortality Risk in Patients with Peripheral Artery Disease: A Systematic Review and Meta-Analysis

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Association of Inflammatory Biomarkers with Morbidity and Mortality Risk in Patients with Peripheral Artery Disease: A Systematic Review and Meta-Analysis

Abstract

Peripheral arterial disease (PAD) is a manifestation of systemic atherosclerosis, which might progress due to inflammation. This systematic review assessed the association of specific inflammatory biomarkers with morbidity and mortality in PAD patients. MEDLINE and EMBASE databases were systematically searched for studies assessing evidence between inflammatory biomarkers and morbidity and mortality risks in PAD patients. Results were reported as Hazard Ratios (HR), Odds Ratios (OR), or mean and standard deviation. Effect estimates for high-sensitivity C-reactive protein (hs-CRP) were pooled using a random-effects model and respectively displayed in forest plots. The study reviewed a total of 6941 records, out of which 26 studies were included for qualitative synthesis, and 9 for quantitative synthesis. A total of 4673 patients were analyzed in the meta-analysis. Elevated baseline IL-6 levels were consistently linked to poor outcomes, including loss of patency and composite endpoints. TNF-α and related biomarkers were associated with adverse outcomes like mortality and patency loss. Elevated IL-1 levels predicted worse cardiovascular outcomes, and IL-1 receptor antagonist levels indicated recurrence or new lesions post-surgery. Hs-CRP was statistically significantly associated with all-cause mortality and MALE in the pooled analysis. The study highlights the ability of inflammatory biomarkers to predict clinical outcomes in PAD patients. The strength of these associations varies based on the specific biomarker and clinical context.

Keywords: Chronic Limb-Threatening Ischemia, Intermittent Claudication, Interleukins, Tumour Necrosis Factors, C-Reactive Protein

Word count:4615

Abbreviation list: CI: Confidence Interval, CRP: C-Reactive Protein, CV: Cardiovascular, HR: Hazard Ratio, hs: High-sensitivity, hs-CRP: High-sensitivity C-Reactive Protein, IFN-γ: Interferon-gamma, IL: Interleukin, IL-1RA: Interleukin-1 Receptor Antagonist, IL-6: Interleukin-6, IL-8: Interleukin-8, IL-10: Interleukin-10, IL-18: Interleukin-18, MALE: Major Adverse Limb Events, MACE: Major Adverse Cardiovascular Events, MACLE: Major Adverse Cardiovascular and Limb Events, NHLB: National Heart, Lung, and Blood Institute, NO: Nitric Oxide; NOS3: Endothelial Nitric Oxide Synthase, OR: Odds Ratio, PAD: Peripheral Arterial Disease, TNF-α: Tumor Necrosis Factor-alpha, TNFR1: Tumor Necrosis Factor Receptor 1.

Introduction

Peripheral arterial disease (PAD) is a clinical manifestation of systemic atherosclerosis [1] that generally involves the lower extremity arteries. The prevalence of PAD appears to be increasing in low- and middle-income countries [1], with conventional cardiovascular risk factors such as diabetes, aging, smoking, high cholesterol, and high blood pressure likely playing a major role in this changing pattern [2]. PAD affects over 27 million people in Europe and North America [3] and is a major contributor to limb loss and death worldwide [4].

Atherosclerosis is an inflammatory-related disease [5]. Research has shown that the development of atherosclerosis is driven by an inflammatory process [6-8]. Thus, elevated cytokine levels may accelerate the progression of systemic atherosclerosis [9].

This implies that an inflammatory response to various triggers (such as smoking, dyslipidemia, hypertension, barotrauma, and shear stress) could contribute to the development and progression of peripheral artery disease (PAD), as well as influence outcomes of leg revascularization, the risk of leg amputation, and patient survival [10-18]. Therefore, there is interest in using blood biomarkers to monitor prognosis and treatment efficacy in PAD patients. Based on current evidence, we have identified a panel of biomarkers that may be more closely connected with each other and correlated with the risk of developing worse outcomes in patients with PAD.

Tumor necrosis factor (TNF)-α plays a particular role during PAD due to its potential in both vascular and skeletal muscle pathophysiology [5]. TNF-α is known for its involvement in smoking-related inflammation and vascular damage [19,20], as well as in regulating normal vascular tone by affecting the availability of vascular nitric oxide, the repair of damaged endothelium, vasculogenesis, angiogenesis, and the function of circulating angiogenic cells in response to adiponectin [16,21]. Interleukin-6 (IL-6) is a pleiotropic cytokine involved in various actions relating to inflammation, host defense, and tissue injury [22]. High-sensitivity C-reactive protein (hs-CRP) is a marker of inflammation derived from leukocytes in response to IL-6 stimuli [23]. High levels of IL-6, C-Reactive Protein (CRP), and TNF-α contribute to plaque formation [24]. Additionally, C-reactive protein (CRP) has been associated with restenosis after percutaneous transluminal angioplasty of lower limb arteries [25]. Interleukin (IL)-1 is a potent pro-inflammatory cytokine involved in the earliest steps of the atherosclerotic process [26]. The pro-inflammatory cytokine IL-1 is a significant driver of inflammation, with well-established detrimental effects in various preclinical models of systemic inflammatory disease [11]. It has been proposed that IL-1ß may play an instrumental role in the pathogenesis of atherosclerosis by stimulation of the vascular smooth muscle cells [27]. Interleukin-1 (IL-

1), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) have been demonstrated to contribute not only to the formation of atherosclerotic plaques but also to the development of neointima in arterial segments affected by restenosis, which is often regarded as a key factor in reconstructive vascular failure [9,28].

Moreover, IL-8 is a powerful neutrophil chemotactic cytokine (chemokine) involved in inflammation and host defense [29-31]. Produced by various cell types, it can be found in any tissue and is released during infections, ischemia, trauma, and other disruptions to tissue homeostasis. IL-8 is likely the primary driver of local neutrophil accumulation, making it a potential biomarker for predicting peripheral artery disease (PAD) [11]. IL-10 is an important immunoregulatory cytokine that inhibits the production of proinflammatory cytokines, such as interferon-γ, IL-2, IL-3, and TNF-α, which are produced by Th1 cells, B cells, macrophages, and natural killer cells [32]. Although evidence on the anti-inflammatory effects of IL-10 in neointimal thickening and restenosis following arterial injury is limited, some studies suggest that IL-10 plays an important and essential role in the suppression of intimal hyperplasia, restenosis, and atherosclerosis [33].

Other cytokines contribute to vascular inflammation as well. Mallat et al. highlighted the presence of IL-18 in plaque macrophages and observed increased IL-18R expression in both plaque macrophages and endothelial cells within human atherosclerotic plaques. There were markedly higher levels of IL-18 mRNA in unstable symptomatic plaques compared to stable asymptomatic ones [34]. The role of IFN-Y in the pathophysiology of atherosclerosis is unquestionably intricate and the pro-atherogenic versus anti-atherogenic nature of the cytokine has long been a subject of debate. Nevertheless, research from mouse models of the disease that are deficient in IFN-Y signaling points towards a largely pro-atherogenic role [35].

This systematic review and meta-analysis aims to assess the association between selected inflammatory biomarkers and morbidity and mortality risk in PAD patients.

Methods

The study protocol was registered in PROSPERO (PROSPERO ID: CRD42024530001) per standard reporting conventions. This manuscript was written using PRISMA guidelines.

Data sources and search strategy

A MEDLINE and EMBASE database search was performed to find evidence up to the 28th of March 2024 on the association between inflammatory biomarkers and morbidity and mortality risk in PAD patients (Supplemental Table 1S).

Study selection

Two reviewers independently screened search records for inclusion (FM, MFM), and another checked decisions (JRN). Titles and abstracts were screened at this stage and relevant studies were selected for full-text analysis.

Included studies were prospective cohorts, retrospective cohorts, case-control studies, or post hoc analyses of randomized controlled studies. Other inclusion criteria comprised 1) patients aged 18 years or older; 2) with a diagnosis of peripheral artery disease; 3) a minimum of 1 of the pre-specified biomarkers measured in the peripheral blood previous to the occurrence of the outcomes of interest; 4) describe at least one of the outcomes of interest (all-cause mortality, cardiovascular mortality, stroke, myocardial infarction, congestive heart failure, major adverse cardiovascular events - MACE, untreated loss of patency of a revascularization procedure, re-intervention on a revascularization procedure or recurrence of disease, major amputation, major adverse limb events

– MALE, major adverse cardiovascular and limb events - MACLE); 5) describe the association of at least one measured biomarker with one outcome of interest. The pre-specified biomarkers included: high-sensitivity (hs) CRP, TNF-alpha, interferon-gamma inducing factor, IL-1β, IL-6, IL-8, IL-10, and IL-18.

Exclusion criteria encompassed 1) studies with less than 20 patients; 2) studies published in abstract form; 3) studies not published in the English language; 4) studies published before 1/January/2014; 5) duplicate manuscripts; and 6) case-reports and case series.

Data extraction and quality assessment

Two reviewers (MFM, FM) blindly and independently checked the full texts, decided on the inclusion of individual studies, and extracted data about study characteristics, inflammatory biomarkers, and events. A senior reviewer decided on disagreements between reviewers (JRN). Extracted data included effect measures or descriptives of the pre-specified included outcomes, presented as Hazard Ratios (HR), Odds Ratios (HR), or mean and standard deviation. Ninety-five percent Confidence Intervals (CI) were reported when existent.

Concerning qualitative assessment, the National Heart, Lung, and Blood Institute (NHLB) Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies was used to assess the risk of bias, adequate reporting and quality of statistical analysis of observational cohorts and cross-sectional studies. Evaluated bias domains included bias related to the research question, study population, sample size, exposure, outcomes, loss to follow-up, and confounding variables.

Data analysis

Assessment of reporting bias was performed with the Egger's regression test and respective funnel plot. Heterogeneity was evaluated through Q statistic-related measures of variance (Tau^2, I^2, Chi-squared test), and a random-effects model with an inverse variance method was preferred to compute estimates for the summary effect. Forest plots were used to display results from the meta-analysis, where the measure of effect for each study is represented by a square and the respective areas are proportional to study weight.

A *p* value inferior to 0.05 was considered statistically significant for all analyses. Computations were conducted using Review Manager 5.3 and Stata 16.1.

Results

A total of 6941 records were identified through database searching, of which 4282 were kept after duplicates were removed (Supplemental Figure 1S).

Ninety-eight publications were assessed for eligibility through their full texts, with 26 manuscripts selected for qualitative synthesis. Due to heterogeneity between studies, only manuscripts with hs-CRP as exposure were included in the quantitative synthesis. Of those, three publications were excluded from the analysis since the outcome of interest was compared without using a longitudinal effect measure. One additional study was not included in the quantitative synthesis as it presented myocardial infarction and stroke as outcomes, which were not evaluated in any other studies with hs-CRP as exposure. When more than one estimate was presented in a given study, the adjusted estimates for hs-CRP as a continuous variable were preferred for quantitative synthesis. Supplemental figure 1S depicts the study selection process.

Included research papers were published between 2017 and 2023, with a wide range of locations (US, Brazil, Japan, China, South Korea, and several countries in Europe). All papers were observational and based on a prospective or retrospective cohort (Supplemental Table 2S), with one of the included studies being a *post hoc* of a randomized controlled trial. The total number of included patients with PAD for quantitative synthesis was 2283 (all-cause mortality), 1738 (MACE), 356 (MALE), and 296 (revascularization). The majority of included studies presented as prognostic markers TNF-α, IL-6, and hs-CRP. Quantification of hs-CRP was based on immunoassays, although the specific assay varied across studies, with some not providing information on the method or analyzer (Supplemental Table 3). Of the studies included in quantitative synthesis, MACE was defined as 1) composite endpoint of stroke, myocardial infarction, and death [36], and 2) CV death, non-fatal MI, and non-fatal stroke [37]. Revascularization in the meta-analysed studies was defined as 1) reintervention within 12 months of endovascular treatment [38]; and 2) clinically driven target lesion revascularization [39]. Finally, MALE was defined in the studies selected for quantitative analysis as 1) a composite endpoint of limb amputation, target vessel revascularization, and disease progression [36]; 2) a composite of clinically driven target vessel revascularization and target limb major amputation, and all-cause death [40].

All studies had a mean age above 60 years (61–74.5), even though gender distribution varied significantly across studies (51.2%–91% of males). Diabetes prevalence represented 25.1% to 100% of each cohort's patients (Table 1). Selected research papers included both surgical and non-surgical settings, with the latter including both outpatient and inpatient contexts (Table 2). Rutherford's class varied across included studies, although most patients did not present prior PAD revascularization (Table 3).

The risk of bias is represented in Fig. 2S (supplementary materials). All included studies, with the exception of Akkoca et al, did not present sample size justification or power description, although effect estimates were provided in almost all studies – for this reason, domain 5 of the NHLB tool was classified as high-risk of bias for most studies. Information was lacking in most research papers on the blinding of outcome assessors, as well as on the participation rate of eligible participants. Some included papers used adjusted time-dependent analysis, and thus were considered as having low risk in domain 14, even though sensitivity or competing risks analyses (e.g., Fine and Gray's model) were not performed for competing risks (Figure 2S). Overall, all selected studies were considered as having a low risk of bias as per the NHLB tool. Tables 4 and 5 present, for each study, outcome definitions, effect measures, and follow-up times.

IL-6

Loss of Patency/Restenosis

Loss of patency or restenosis was evaluated for IL-6 in 4 of the studies included for qualitative synthesis [11,41-44]. All of these studies had pre and postoperative samples in which IL-6 was quantified and were performed in a surgical context where the type of surgery was either lower limb angioplasty [11,41-44], lower limb bypass and lower limb angioplasty [38], or midfoot amputation and lower limb angioplasty [43]. Follow-up varied between 6 and 22 months and a higher level of pre/postoperative IL-6 was identified as a risk factor for loss of patency in 3 studies [42-44]. Conversely, 2 studies did not suggest an association of pre/postoperative IL-6 levels with restenosis [11,41].

Composite Endpoints

Four studies included in the qualitative synthesis tested for an association of IL-6 levels

with composite endpoints: amputation-free survival as a combined endpoint [45], MACE [24,46], MACLE [47], and MALE [24,46]. Definitions of MACE and MALE varied slightly between studies (Table 4). The clinical context was either non-surgical [47], lower limb angioplasty [24,46], or non-surgical or iliofemoral endarterectomy [40]. Studies presented either baseline/preoperative samples [24,45,47] or both pre and postoperative samples [46], with a follow-up range from 12 to 39 months. IL-6 levels were significantly associated with composite outcomes in all four studies [24,45-47].

TNF-α, TNFR1, TNF-α indexed to fat mass, TNF-α indexed to visceral adiposity

Loss of Patency/Restenosis

Loss of patency was evaluated in 2 out of the 4 studies included in qualitative synthesis that presented data on TNF-α or related biomarkers. Both studies suggested an association between pre/postoperative TNF-α values and loss of patency (revascularization failure) [44] and occlusion of the vein graft [43], in the context of either lower limb bypass and angioplasty [44] or lower limb angioplasty and midfoot amputation [43]. Additionally, restenosis was not associated with pre/postoperative TNF-α levels in the context of lower limb angioplasty for the 2 studies included for qualitative synthesis that tested for this hypothesis [11,41]. Follow-up ranged between 6 to 22 months for these endpoints.

Revascularization

Preoperative blood concentration of TNF-α indexed to fat mass and to visceral adiposity score was higher in patients who needed re-intervention at 12 months of follow-up after lower limb angioplasty. However, after adjustment for covariates, the biomarker was no longer a predictor of the outcome [5].

Major Amputation

One of the included studies suggested an association between preoperative TNFR1 levels in the context of major lower limb amputation surgery, and major amputation at follow-up (median 84 months), defined as an amputation above the ankle, including leg or thigh amputation [48].

All-Cause Mortality

Caicedo et al was the only selected study that evaluated the association of TNF-α with all-cause mortality – in an outpatient setting, a concentration of baseline plasma TNF-α ≥ 8.1 pg/mL was significantly associated with cumulative mortality at 12 months.

Composite Endpoints

MACE [5,24,46] and MALE [24,46] at 12 months of follow-up were significantly associated with baseline elevated concentrations of TNF-α in all included studies that tested for these hypotheses. Definitions of MACE and MALE varied slightly across papers (Table 4), although all 3 studies had a clinical context of lower limb angioplasty surgery. Spychalska-Zwolińska et al [5] presented levels of TNF-α indexed to fat mass and to visceral adiposity score.

IL-1, IL-1 receptor antagonist

Two included studies evaluated the association of IL-1 or IL-1 receptor antagonist with outcomes after vascular surgery. Higher baseline IL-1 levels were associated with worse cardiovascular outcomes (MACE and MALE) at 12 months in the context of lower limb angioplasty [24]. Moreover, preoperative IL-1 receptor antagonist (IL-1RA) concentrations predicted recurrence or new lesions at 12 months after lower limb angioplasty or foot surgery [49].

IL-10

Two papers were included for qualitative synthesis with IL-10 measurements – Sega et al [49] reported that higher levels of IL-10 at baseline predicted new lesions or recurrences at 12 months after lower limb angioplasty or foot surgery. However, at 6 months of follow-up in the context of lower limb angioplasty, IL-10 was not associated with restenosis in a different study [11].

IL-8

Two manuscripts evaluated IL-8 in PAD patients. Elevated preop/postoperative levels of the inflammatory biomarker IL-8 were independently associated with amputation-free survival, in either a non-surgical or iliofemoral endarterectomy clinical context at a mean of 39 months of follow-up [45]. Conversely, IL-8 was not associated with restenosis at 6 months in a study with patients submitted to lower limb angioplasty [11].

Hs-CRP

All-cause mortality

All-cause mortality was reported in 5 [40,50-53] out of the 9 selected studies for quantitative analysis. The median or mean follow-up varied between 3.5–10 years and the pooled HR is 1.86 [1.22–2.85] ($p = 0.004$; $I^2 = 84\%$), with a total of 2283 analysed patients (Figure 1). Covariates used in the adjusted models are presented in Table 4S. Publication bias for this outcome was evaluated by a funnel plot and Egger's regression test ($p = 0.0002$), which suggests presence of bias – Figure 2.

Figure 1. Association between hs-CRP and all-cause mortality (forest plot).

Figure 2. Funnel plot depicting absence of publication bias for all-cause mortality and hs-CRP.

MACE

MACE was reported in 2 of the selected studies [36,37] and was defined as a composite of non-fatal MI, non-fatal stroke, and CV mortality [37]; or stroke, MI, and all-cause mortality [36]. A total of 1738 patients were included in the analysis and median follow-up time varied between 25 and 36 months. Covariate adjustment is described in Table 4S. The pooled estimate was HR 1.56 [0.81–2.99] (p=0.18; $I^2 = 0\%$) and the respective forest plot is represented in Figure 3.

Figure 3. Association between hs-CRP and MACE (forest plot).

MALE

MALE was analysed in 2 [36,40] studies selected for quantitative synthesis. The median follow-up varied between 36 months to 3.5 years, with a total of 356 analysed patients. The pooled HR is 2.40 [1.03–5.63] ($p = 0.04$; $I^2 = 65\%$), (Figure 4, covariates presented in Table 4S). MALE was defined either as the composite endpoint encompassing limb amputation, target vessel revascularization, and disease progression (occlusion of the treated vessel) [36], or the composite of clinically driven target vessel revascularization, target limb major amputation, and all-cause death [40].

Figure 4. Association between hs-CRP and MALE (forest plot).

Revascularization

Revascularization was reported in 2 studies [38,39]. A total of 296 patients were included in the quantitative analysis and median follow-up time ranged between 12 and 21 months. Covariate adjustment is described in Table 4S. The pooled estimate was HR 1.54 [0.73–3.25] ($p = 0.26$; $I^2 = 87\%$) and the respective forest plot is represented in Figure 5.

Figure 5. Association between hs-CRP and revascularization (forest plot).

Discussion

The study reviewed a total of 6941 records, out of which 26 studies were included for qualitative synthesis, and 9 for quantitative synthesis. A total of 4673 patients were analyzed in the meta-analysis. Elevated baseline IL-6 levels were consistently linked to poor outcomes, including loss of patency and composite endpoints. TNF-α and related biomarkers were associated with adverse outcomes like mortality and patency loss. Elevated IL-1 levels predicted worse cardiovascular outcomes, and IL-1 receptor antagonist levels indicated recurrence or new lesions post-surgery. High-sensitivity C-reactive protein (hs-CRP) was statistically significantly associated with all-cause mortality and MALE in the pooled analysis. The study's overall findings demonstrated the predictive power of inflammatory biomarkers for clinical outcomes in patients with PAD, with variations in the strength of these associations depending on the specific biomarker and clinical context.

Peripheral artery disease (PAD) is a condition characterized by atherosclerotic obstruction primarily affecting the arteries supplying the lower extremities and is increasingly prevalent [54]. PAD is a major cause of morbidity and has a global impact, with millions of patients affected [1]. Atherosclerosis itself is a chronic inflammatory process, and elevated cytokine levels have been shown to exacerbate the progression of this disease [5,9]. Inflammation plays a crucial role in the pathophysiology of atherosclerosis and its associated complications, which includes PAD. Elevated levels of inflammatory biomarkers have been linked to adverse outcomes in cardiovascular diseases, reflecting the systemic inflammatory burden present in these patients [10-18]. Additionally, patients with PAD are at increased risk of adverse cardiovascular events, limb ischemia, and mortality [4,55]. Percutaneous transluminal angioplasty with stent placement have replaced open vascular surgery over the past few decades, but restenosis emerges as the main cause

of treatment failure [56]. Nearly half of patients with an initially successful balloon angioplasty will present with a restenosis in the first 6 months after intervention and will need some kind of reintervention [25,57]. Therefore, prior to the procedure, it would be key to identify patients who are at a higher risk of complications. Previous research has shown that the pre-procedural inflammatory state is related to an increased risk for restenosis after coronary angioplasty [57]. In this manuscript, higher levels of pre/postoperative IL-6 or TNF-α were identified as risk factors for loss of patency, although not for restenosis. Similarly, IL-10 and IL-8 levels were not associated with restenosis in one of the included studies. Conversely, IL-1RA and IL-10 concentrations predicted recurrence or new lesions at 12 months of follow-up.

PAD patients have shown more widespread atherosclerosis, and the risk of MACE appears to be high in PAD patients [58]. As a result, understanding the association between inflammatory biomarkers and clinical outcomes in PAD is crucial for risk stratification and targeted therapeutic interventions [2,59-61]. The identified biomarkers, including hs-CRP, IL-6, TNF-α, IL-1, IL-10, and IL-8, are known mediators of inflammation and are implicated in the pathogenesis of atherosclerosis and its complications, which underlies PAD [62]. Elevated levels of these biomarkers reflect the inflammatory response within atherosclerotic plaques, promoting plaque instability, endothelial dysfunction, and thrombosis. These processes contribute to the progression of PAD and increase the risk of adverse cardiovascular and limb-related events [63]. The association of these biomarkers with restenosis, loss of patency, revascularization, and major adverse events underscores the role of inflammation in the pathophysiology of PAD and its complications [1,46,57,64-66]. In this manuscript, TNF-α levels were not associated with revascularization after covariate adjustment in one of the included studies. This endpoint was also not predicted by hs-CRP levels in the pooled analysis (non-significant). Elevated

levels of hs-CRP, an acute-phase reactant, are indicative of systemic inflammation and are associated with endothelial dysfunction and atherosclerotic plaque instability [67]. HsCRP could have a negative influence on angiogenesis as well [68]. IL-6, known for its role in chronic inflammation, further amplifies the inflammatory response, promoting atherogenesis and thrombosis [67]. IL-6 inhibits nitric oxide production in endothelial cells, with consequent oxidative stress, and this effect is directly dependent on TNF-activity [69]. TNF-α, a key proinflammatory cytokine, acts synergistically with IL-1 to induce endothelial activation and vascular smooth muscle cell proliferation, contributing to atheroma formation and plaque vulnerability, all of which are critical for PAD progression [27]. TNF- α can affect the activity of NOS3, reducing the level of nitric oxide (NO), facilitating endothelial dysfunction, and altering the regeneration process [70,71]. On the other hand, interleukin-10 is an anti-inflammatory cytokine and plays a regulatory role in dampening the proinflammatory response, but its dysregulation in cardiovascular disease may contribute to ongoing inflammation and disease progression [33]. IL-8, a potent chemoattractant, is involved in leukocyte recruitment and endothelial activation, exacerbating atherosclerotic processes and promoting plaque instability [29].

In PAD there is a scarcity of tools for the early diagnosis, progression and prognosis evaluation, resulting in suboptimal therapeutic interventions. Non-invasive circulating serum biomarkers may be predictive effective tools in this setting. Therefore, the combination of a panel of biomarkers and conventional cardiovascular risk factors could be useful to obtain more accurate prediction algorithms [24,72,73]. The correlations observed between inflammatory biomarkers and adverse clinical outcomes in peripheral arterial disease (PAD) patients in this manuscript may have clinical implications for treatment and prognosis. According to these results, inflammatory biomarkers may be useful instruments for risk stratification, allowing medical professionals to identify high-risk

patients who are more likely to experience complications including MACE, MALE, loss of patency, or death. These patients might benefit from tailored interventions meant to reduce these risks and enhance patient conditions. In other words, risk stratification models in PAD patients are necessary to predict future cardiovascular events and death, and biomarker discovery is a first step to improve risk stratification in PAD [47,74,75]. Moreover, the specific targeting of inflammatory pathways has been considered a promising approach for the treatment of cardiovascular disease [76-80]. Hs-CRP has been proposed as a potential target for treatment [81]. Statin therapy mitigates the elevated risk of cardiovascular events in PAD and enhances walking performance, likely due to its anti-inflammatory effects [82]. Ridker et al has shown that the intake of a monoclonal antibody directed against IL-1, used in clinical practice for treatment of rheumatological diseases, significantly reduced the risk of recurrence of cardiovascular events in patients with history of myocardial infarction and persistently high CRP levels, confirming the close link between inflammation and atherosclerosis [83]. Likewise, previous studies showcased that the use of TNF-inhibitors in patients with a high level of inflammation decreased atherosclerotic CV events similar to the use of IL-6 blockers [84], also improving endothelial dysfunction [85]. Interestingly, Das et al. also found that diet supplementation with grape resveratrol was as sociated with a cytokine-lowering effect [86]. In summary, various agents show potential for targeting inflammation in atherosclerosis [62]. The CANTOS trial supports the concept of anti-inflammatory atheroprotection, revealing that IL-1β inhibition via a monoclonal antibody reduced the risk of MACE by 15% [83]. Hs-CRP might be used to select an optimal cohort to apply anti-inflammatory therapy in PAD patients. Additionally, the use of inflammatory biomarkers might enhance the management of PAD, particularly in guiding decisions - the decision to amputate the affected limb, choosing the type of revascularization, optimizing medical management or referral

to specialized care settings would benefit from improved risk-stratification [87]. This could result in a better-informed strategy that maximizes the selection of surgical candidates, lowers the risk of complications following surgery, and eventually improves long-term outcomes.

The study's strengths lie in its reliability and comprehensive evaluation of variable inflammatory biomarkers and their associations with a wide range of clinical outcomes in PAD patients. A comprehensive and systematic search was conducted, identifying a substantial number of records, with many studies retained after duplicates were removed, ensuring a thorough review process. The study selection was rigorous, ultimately resulting in the inclusion of a smaller number of manuscripts for qualitative synthesis. Most included studies were considered to have a low risk of bias, further supporting the credibility of the findings. The inclusion of a diverse patient population across multiple countries and a longitudinal assessment of outcomes contribute to the robustness of the findings, enhancing its generalizability. Additionally, the study incorporated a determination of blood concentrations of a wide range of biomarkers in patients in both surgical and non-surgical settings, as well as a follow-up period of sufficient length to enable us to observe the effect of hs-CRP, TNF-α, IL-1, IL-6, IL-8, and IL-10 concentrations on the outcomes measured. However, this study has limitations. First, the heterogeneity of the included studies, including differences in study design, patient populations, and interventions, may introduce variability in the results and limit the ability to draw broad conclusions. Second, variations in biomarker measurement methods across studies based on immunoassays with differing methods and analyzers could affect the accuracy and comparability of the findings. Third, potential confounding factors may not have been fully accounted for in the analyses, leading to biased results. Additionally, publication bias was identified for the association of hs-CRP with all-cause mortality, indicating the presented

results might not be the full picture. All included studies had a mean age above 60 years, which may limit the applicability of the findings to younger populations. Information was also lacking in most studies regarding the blinding of outcome assessors and the participation rate of eligible participants, which could introduce biases in outcome assessment and impact the validity of the results. Finally, the generalizability of the findings may be influenced by the specific patient populations and clinical contexts represented in the included studies, reducing their applicability to broader or different settings.

In conclusion, the study provides valuable insights into the associations between inflammatory biomarkers and adverse clinical outcomes in PAD patients. These findings highlight the intricate link between inflammation and the pathophysiology of PAD, emphasizing the potential of inflammatory biomarkers as prognostic indicators. Moving forward, further research is warranted to validate these associations, elucidate underlying mechanisms, and explore the clinical utility of inflammatory biomarkers in guiding personalized management strategies for PAD patients.

Conclusions

The study highlights the ability of inflammatory biomarkers to predict clinical outcomes in PAD patients. The strength of these associations varies based on the specific biomarker and clinical context.

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Declaration of conflict of interest

The authors report no conflict of interest.

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Figure captions:

Figure 1. Association between hs-CRP and all-cause mortality (forest plot).

Figure 2. Funnel plot depicting absence of publication bias for all-cause mortality and hs-CRP.

Figure 3. Association between hs-CRP and MACE (forest plot).

Figure 4. Association between hs-CRP and MALE (forest plot).

Figure 5. Association between hs-CRP and revascularization (forest plot).

Figures

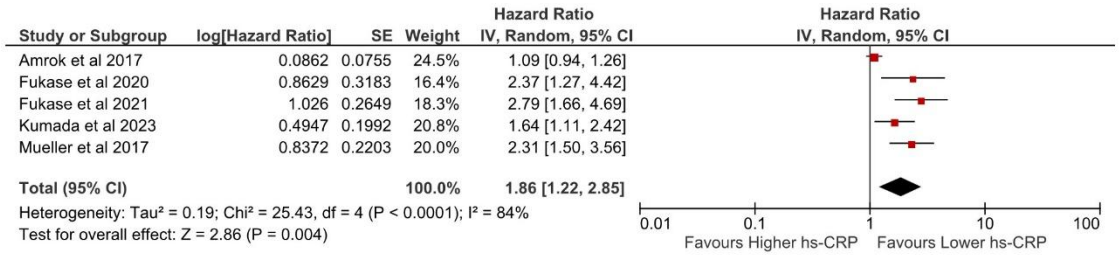

Figure 1. Association between hs-CRP and all-cause mortality (forest plot).

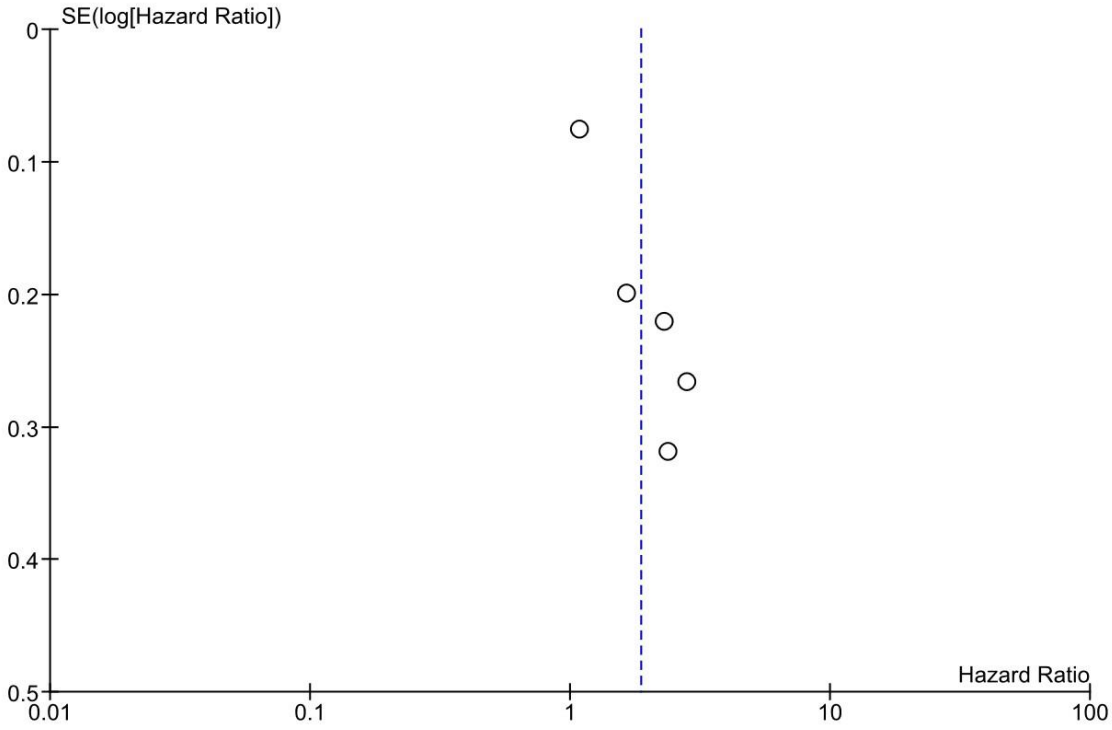

Figure 2. Funnel plot depicting absence of publication bias for all-cause mortality and hs-CRP.

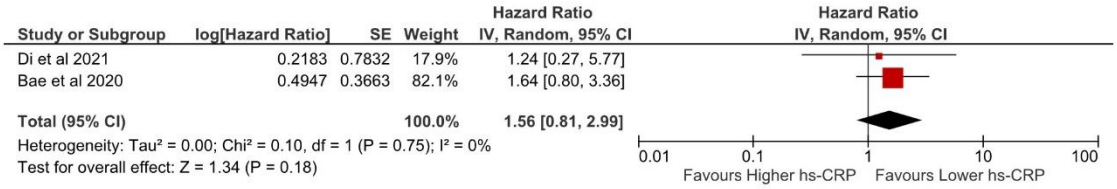

Figure 3. Association between hs-CRP and MACE (forest plot).

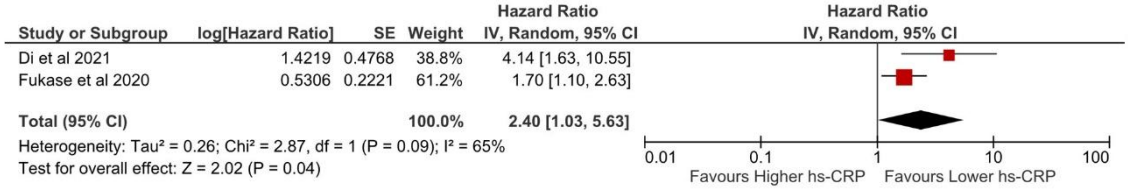

Figure 4. Association between hs-CRP and MALE (forest plot).

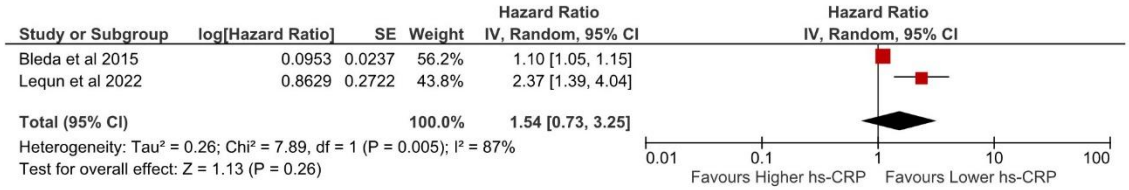

Figure 5. Association between hs-CRP and revascularization (forest plot).

Table 1. Patient characteristics of included studies.

Author	**Sample size**	**Mean age**	**Male n (%)**	**Arterial hypertension n (%)**	**Dyslipidemia n (%)**	**Diabetes n (%)**	**Smoking history n (%)**	**BMI**	**CKD n (%)**
Bleda et al	174	67.7 ± 9.8	94 (77.7)	83 (68.6)	43 (35.5)	73 (60.3)	76 (62.8)	NA	18 (14.9)
Ueki et all	35	74.5 ± 7.4	31 (88.6)	31 (88.6)	26 (74.3)	21 (60)	26 (74.3)	23.0 ± 4.6	NA
Amrock et al	647	67.8 (standard error 0.7)	269 (41.6)	496 (76.6)	197 (30.5)	162 (25.1)	162 (25.1)	28.5 (standard error 0.3)	NA
Mueller et al	487	61-69	71-86%	42-82%	NA	NA	40-58%	25-28	9-34%
Schneider et al	1468	65 ± 11	848 (58)	NA	NA	1468 (100)	153 (10)	31 ± 6	NA
Guimarães et al	27	66.2 ± 10.6	19 (70)	20 (74)	6 (22)	11 (41)	19 (70)	NA	NA

Akkoca et al	67	62.3 ± 9.7	61 (91)	21 (55.3)	NA	17 (44.7)	5 (13.2)	NA	5 (13.2)
Rief et al	2124	69.3 ± 12.0	1266 (59.6)	1731 (81.5)	NA	713 (33.6)	1142 (53.8)	25 (23–28)*	608 (28.5)
Sapienza et al (a)	68	72.79	56 (82.35)	37 (54.4)	NA	NA	43 (63.23)	NA	4 (5.88)
Gremmels et al	108 + 146	66	74 (68.52)	NA	NA	24 (22.22)	26 (24.07)	26.7	NA
Biscetti et al	299	73.0 ± 5.1	153 (51.2)	141 (47.2)	NA	299 (100)	110 (39.5)	30.2 (1.8)	NA
Sapienza et al (b)	37	70 ± 7	31 (83.78)	31 (4)	NA	NA	23 (62)	NA	4 (11)

Spychalskana-zwolińska et al	70	65.51 ± 11.17	40 (57.14)	54 (77.14)	56 (80)	26 (37.14)	25 (35.71)	26.33 ± 4.66	NA
Guo et al	68	73.75	51 (75)	41 (60.29)	27 (39.7)	33 (48.53)	44 (64.70)	22.6	NA
Saenz Pipaon et al	317	70 ± 11	279 (88)	238 (75)	212 (67)	168 (53)	105 (33)	NA	NA
Bae et al	1667	71.0 ± 12.3	132 (65.7)	122 (60.7)	146 (72.6)	70 (34.8)	101 (50.2)	23.4 ± 4.0	NA
Fukase et al (d)	285	72 ± 8	208 (73)	246 (86)	202 (71)	191 (67)	216 (77)	22.9 ± 4.0	152 (53)
Fukase et al (c)	335	72 ± 8	274 (82)	280 (84)	251 (75)	187 (56)	140 (42)	23.1 ± 3.8	119 (36)

Kadoglou et al	168	76 ± 11	141 (83.9)	125 (74.4)	162 (96.4)	68 (36.9)	43 (25.6)	27.98 ± 3.22	NA
Sega et al	92	72.5 (68-78)*	71 (77.2)	NA	NA	85 (93.4)	9 (9.9)	26.68 (24.93-29.30)*	NA
Kremers et al	120	67.7 ± 9.6	70 (58.3)	90 (75)	115 (95.8)	31 (25.8)	53 (44.2)	NA	NA
Caicedo et al	35	71 ± 12.7	28 (79.4)	NA	NA	21 (59)	Intervention group: 31 (88.89); Placebo: 18 (50)	NA	9 (26.5)
Di et al	71	69.7 ± 8.6	Normal hs-CRP: 30	Normal hs-CRP: 24	Normal hs-CRP: 8 (20.0);	Normal hs-CRP: 20	Normal hs-CRP: 25	BMI >25: normal hs-	normal hs-CRP: 1

			(75.00); Abnormal: 24 (77.42)	(60.0); Abnormal: 22 (71.0)	Abnormal: 6 (19.4)	(50.0); Abnormal: 15 (48.4)	(62.5); Abnormal: 18 (58.1)	CRP: 15 (37.5); Abnormal: 13 (41.9)	(2.5); Abnormal: 1 (3.2)
Lequn et al	122	67.2 ± 9.2	104 (85.2)	94 (77)	90 (73.8)	54 (44.3)	86 (70.5) (former smoker)	NA	NA
Nardella et al	264	73.4 ± 8.8	171 (64.77)	204 (77.3)	243 (92.0)	264 (100)	63 (23.8)	26.4 ± 5.0	NA
Kumada et al	800	67 ± 10	54 (66.9)	50 (62.1)	20 (24.5)	51 (63.3)	21 (25.7)	21.2 ± 3.3	NA
Wang et al	117	67 (59-71)*	77 (65.8)	48 (41)	NA	76 (65)	12 (10.3)	NA	NA

Legend: NA – unavailable data; SE – Standard Error; * Median (Interquartile Range).

Table 2. Clinical context of included studies.

Author	Non-surgical outpatient setting	Non-surgical inpatient setting	Main reason for non-surgical hospital admission	Surgery	Type of surgery
Bleda et al	No	No	NA	Yes	Lower Limb Angioplasty
Ueki, et all	No	No	NA	Yes	Lower Limb Angioplasty
Amrock et al	Yes	No	NA	No	None
Mueller et al	No	Yes	NA	No	None
Schneider et al	No	No	NA	Yes	Major Lower Limb Amputation
Guimarães et al	No	No	NA	Yes	Lower Limb Angioplasty

Akkoca et al	Yes	No	NA	Yes	Medical, Open Vascular, Or Endovascular Intervention
Rief et al	No	No	NA	Yes	NA
Sapienza et al (a)	No	No	NA	Yes	Lower Limb Bypass and Lower Limb Angioplasty
Gremmels et al	No	Yes	Severe infra-popliteal PAD, defined as severe intermittent claudication (Fontaine IIB), ischemic rest pain (Fontaine III) or non-healing ischemic ulcers (Fontaine IV) and ineligibility for angioplasty or bypass surgery, as well as an Ankle-Brachial Index (ABI) ≤ 0.6	Yes	Iliofemoral Endarterectomy
Biscetti et al	No	No	NA	Yes	Lower Limb Angioplasty

Sapienza et al (b)	No	No	NA	Yes	Lower Limb Angioplasty and Midfoot Amputation
Spychalskanazwolińska et al	No	No	NA	Yes	Lower Limb Angioplasty
Guo et al	No	No	NA	Yes	Lower Limb Angioplasty
Saenz Pipaon et al	Yes	No	NA	No	None
Bae et al	No	No	NA	Yes	Percutaneous Coronary Intervention
Fukase et al (d)	No	No	NA	Yes	Lower Limb Angioplasty
Fukase et al (c)	No	No	NA	Yes	Lower Limb Angioplasty
Kadoglou et al	No	No	NA	Yes	Lower Limb Angioplasty

Sega et al	No	No	NA	Yes	Lower Limb Angioplasty and Foot Surgery
Kremers et al	Yes	No	NA	No	None
Caicedo et al	Yes	No	NA	No	None
Di et al	No	No	NA	Yes	Lower Limb Angioplasty
Lequn et al	No	No	NA	Yes	Lower Limb Angioplasty
Nardella et al	No	No	NA	Yes	Lower Limb Angioplasty
Kumada et al	No	No	NA	Yes	Lower Limb Angioplasty or Bypass Surgery

Wang et al	No	No	NA	Yes	Lower Limb Angioplasty

Legend: NA – unavailable data

Table 3. Patient cardiovascular symptoms and background.

Author	ABI	Rutherford (R)/Fontaine	CAD n (%)	Prior PAD revascularization
Bleda et al	NA	R 3-5	36 (29.8) (prior MI)	No
Ueki et all	0.69	R II: 12-34.3%; III: 23- 65.7%	NA	NA
Amrock et al	0.77 (SE 0.007)	NA	NA	NA
Mueller et al	0.56-0.70	R 4-6: 7-40%	28-60% (CAD, cerebrovascular disease or both)	33-51% vascular surgery, percutaneous transluminal angioplasty with or without stenting, or amputation.
Schneider et al	NA	NA	NA	129 (9%) (lower limb revascularization and/or lower limb amputation)
Guimarães et al	NA	R 3: 11 (41%); R 4: 3 (11%); R 5: 13 (48%)	NA	NA

Akkoca et al	0.54 ± 0.2	R 1-5	15 (39.5)	No
Rief et al	NA	NA	746 (35.1)	NA
Sapienza et al (a)	0.355	Rutherford Grade III category 5-6 and moderate to high risk of limb amputation (clinical stages 3-4 according to Wound Ischaemia foot Infection—WIfI—classification)	20 (29.41)	NA
Gremmels et al	0.53 (0.31)	R 3/4/5/6: 7%/36%/61%/4%; Fontaine class IIB/III/IV: 7%/36%/65%	NA	9 (8.3%) (major amputation)
Biscetti et al	0.34 (0.05)	R 4: 142 (47.5%); R 5: 157 (52.5%)	140 (46.8)	NA
Sapienza et al (b)	0.4 ± 0.1	R III category 5	12 (32)	NA
Spychalskanazwolińska et al	NA	NA	NA	NA
Guo et al	0.515	R < V	24 (35.29)	No

Saenz Pipaon et al	0.39-0.67	Fontaine II-IV	NA	No
Bae et al	ABI ≤ 0.9: 11.3%	NA	1667 (100)	No
Fukase et al (d)	0.64 ± 0.16	R 2-6	146 (52)	No
Fukase et al (c)	0.68 ± 0.15	R 2/3: 166 (50%) / 169 (50%)	NA	No
Kadoglou et al	0.48 ± 0.09	NA	NA	NA
Sega et al	NA	R 5-6	42 (45.7)	NA
Kremers et al	ABI <0.9:	R 1: 9(7.5%); R2: 34(28.3); R3: 45 (37.5); R4: 32(26.7%)	39 (32.5) (prior MI)	84 (70%)

Caicedo et al	0.23 ± 0.23	R 5-6: 70.6%	NA	No
Di et al	NA	R ≥ 3	Normal hs-CRP: 8 (20.0); Abnormal: 5 (16.1)	NA
Lequn et al	NA	R 2-6	40 (32.7)	NA
Nardella et al	NA	R 3: 54 (20.4%); R 5: 108 (40.9); R 6: 42 (15.9%)	117 (44.3)	NA
Kumada et al	0.62 (0.45-0.79)*	NA	51 (63.5)	NA

Wang et al	NA	R 3–6	NA	No

Legend: ABI-ankle brachial index; CAD- Coronary Artery Disease; CABG- coronary artery bypass grafting; MI – Myocardial Infarction; NA – unavailable data; PCI- Percutaneous coronary intervention; PAD – Peripheral Artery Disease; NA – unavailable data; R – Rutherford classification of chronic peripheral artery disease; SE – Standard Error. *Median (Interquartile Range).

Table 4. Outcome definitions and summary of results.

Author	Results	Association (No association; Risk Factor; Protective Factor)	Time of measurement	Outcome(s)	Outcome Definitions
Bleda et al	In the multivariate analysis, preoperative hs-CRP levels were the only independent predictors of new revascularization events	Risk Factor	Preoperative	Reintervention	Reintervention within 12 months of endovascular treatment
Ueki, et all	EVT significantly increases IL-6, MCP-1, and TNF-α levels in the ischemic leg, but this effect is not associated with a higher rate of in-stent restenosis.	No association	Preoperative and Postoperative	Untreated loss of Patency	Rate of in-stent restenosis after endovascular treatment
Amrock et al	hs-CRP was not predictive of either all-cause mortality or CV mortality in those with PAD	No association	No Surgery/ Intervention	All-Cause Mortality and Cardiovascular Mortality	ICD 10 codes 100 to 199, from the date of interview to either date

					of death or December 31st 2011
Mueller et al	Hs-CRP was independently associated with all-cause mortality in non-diabetic patients <75 years	Risk Factor	No Surgery /Intervention	All-Cause Mortality	Death occurring during the observation period
Schneider et al	Risk factors associated with minor versus major amputation, including biomarkers such as TNFR1, should be considered differently in patients with T2D.	Risk Factor	Preoperative	Major Amputation	Minor amputation was defined as an amputation below ankle, including toes or transmetatarsal amputation. Major amputation was defined as an amputation above ankle, including leg or thigh amputation
Guimarães et al	This study demonstrated a significant increase in anti-inflammatory TGF-β and	No association	Preoperative and Postoperative	Untreated loss of Patency	Recurrence of symptoms associated with

	IL-10 and a decrease in proinflammatory cytokines IL-1β, IL-6, IL-8, and TNF-α at 6 months of follow-up, but no inflammatory marker was independently identified as a risk factor for in-stent restenosis.				decreases in the ankle-brachial index > 0.15 and duplex ultrasound scan showing an incremental increase in the peak systolic velocity ratio of > 2.5-fold between the adjacent arterial segments were criteria consistent with a significant restenosis. A patient was confirmed to have developed restenosis if there was greater than 50% luminal

					narrowing in the angiography images.
Akkoca et al	hs-CRP levels were significantly higher in the group with MACLE events versus no events.	Risk Factor	Preoperative	MACLE	Cardiovascular death, atherothrombotic stroke, acute coronary syndrome (unstable angina pectoris, without Q-wave acute myocardial infarction, and with Q-wave acute myocardial infarction) and major amputation (amputation of the lower limb above the ankle)

Rief et al	Higher hsCRP values were significant predictors for MI and stroke.	Risk Factor	Preoperative	Stroke and Myocardial Infarction	-
Sapienza et al (a)	Elevated plasma levels of VCAM-1, ICAM-1, IL-6, and TNF-α during follow-up were strongly related to impaired wound healing and/or revascularisation failure.	Risk Factor	Preoperative and Postoperative	Untreated loss of Patency	Vein graft or endovascular failure and wound healing defined as complete epithelialisation without adverse complications during follow up
Gremmels et al	Biomarkers were associated with the combined endpoint at follow-up.	Risk Factor	NA	Amputation-free survival as a combined endpoint	Amputation through or above the ankle joint, at 1 year after inclusion

Biscetti et al	Elevated OPG, TNF-α, IL-6, and CRP levels at baseline correlate with worse vascular outcomes in patients with diabetes, PAD, and CLTI undergoing an endovascular procedure.	Risk Factor	Preoperative and Postoperative	MALE and MACE	The major adverse limb events (MALE) outcome was defined as a composite of acute limb ischemia, major vascular amputations (above the ankle), and limb-threatening ischemia leading to urgent revascularization. The MACE outcome was defined as composite of myocardial infarction, stroke, and

					cardiovascular death.
Sapienza et al (b)	Postoperative high levels of TNF-α, IL-6, and MMP-2 and-9 were predictive for wound healing failure at 3, 6, and 9 months, respectively. Furthermore, the subgroup of patients who experienced occlusion of the vein graft during follow up had a significant increase of MMP-2, -9, IL-6, and TNF-α at 3, 6, and 9 months, respectively.	Risk Factor	Preoperative and Postoperative	Untreated loss of Patency	Wound healing failure and occlusion of the vein graft
Spychalskanazwolińska et al	TNF-α indexed to fat mass and to visceral adipose tissue was higher in patients who underwent reintervention (TLR) and MACE. However, in multifactorial analysis, the association was lost.	Risk Factor	Preoperative	Reintervention and MACE	MACE was defined as the occurrence of all-cause death, all-cause readmission, a cardiovascular event, or in-stent

					restenosis during the follow-up period; reintervention (TLR) was defined as the recurrence after enrolment to the study of intermittent claudication or acute limb ischemia of the stented limb associated with a decrease in ABI in comparison to the values obtained after the first endovascular procedure, and which

					necessitated further intervention
Guo et al	Interleukin-6 is a powerful independent predictor of intermediate-term outcomes for stenting of the femoropopliteal artery.	Risk Factor	Preoperative and Postoperative	Untreated loss of Patency	Restenosis was defined as a proximal systolic peak flow velocity ratio >2.4 based on duplex ultra-sound, which was considered indicative of a stenosis of greater than 50%.
Saenz Pipaon et al	Biomarkers were associated with amputation and CV death.	Risk Factor	No Surgery/ Intervention	Amputation; Amputation and CV death	Major Amputation; Amputation and CV death
Bae et al	PAD patients with high hs-CRP (≥ 3.0 mg/L) had the greater risk of 3-year MACE	No association	Preoperative	MACE	MACE: CV death, non-fatal MI, non-fatal stroke

	than PAD patients with low hs-CRP (< 3.0 mg/L), although not statistically significant.				
Fukase et al (d)	Higher hs-crp was a strong independent predictor of all-cause death.	Risk Factor	Preoperative	All-Cause Mortality and MALE	MALE defined as a composite of clinically driven target vessel revascularization (CD-TVR) and target limb major amputation, and all-cause death.
Fukase et al (c)	Higher hs-CRP levels were significantly associated with all-cause death and cardiovascular-related in patients with intermittent claudication.	Risk Factor	Preoperative	MACLE, All-Cause Mortality, Cardiovascular Mortality, Stroke, Myocardial Infarction,	MACLE was defined as a composite of cardiovascular-related death, non-fatal acute coronary syndrome, stroke, clinically driven target vessel

				Reintervention, Limb ischemia	revascularization (CD-TVR) and development to limb ischemia, and all-cause death. CD-TVR was defined as re-intervention at the target vessel when symptoms occurred, and significant stenosis was detected by duplex ultrasonography or when the ankle-brachial index value markedly decreased. Development to limb ischemia was assumed when it was newly

					detected the presence of ischemia, rest pain and tissue loss in the patients with intermittent claudication.
Kadoglou et al	hsCRP was higher in patients with PAD and with MACE vs non-MACE.	Risk Factor	Preoperative	MACE	MACE: acute coronary events and peripheral artery restenosis requiring re-revascularization (either endovascular or open surgery)
Sega et al	The occurrence of new lesions or recurrences at 12 months were positively associated with the serum levels of IL-10 and IL1RA.	Risk Factor	Preoperative	new lesion or recurrence	A new lesion was defined as the occurrence of a new wound at a site other

					than the treated DFU; a recurrence was defined as a wound occurring at the same site of the treated DFU after a successful healing.
Kremers et al	IL6 was positively predictive of MACLE	Risk Factor	No Surgery/ Intervention	MACLE	Composite endpoint of myocardial infarction, stroke, acute limb ischemia, elective percutaneous coronary intervention or coronary artery bypass grafting and all-cause mortality during one year of follow-up.

Caicedo et al	A value of plasma TNF-α ≥ 8.1 pg/mL was significantly associated with cumulative mortality at 12 months.	Risk Factor	No Surgery/ Intervention	All-Cause Mortality	-
Di et al	Abnormal hs-CRP levels were significantly correlated with the occurrence of MALE. No significant association was found between hs-CRP level and MACE.	MALE: Risk Factor; MACE: No association	Preoperative	MALE and MACE	MALE was defined as the composite endpoint encompassing limb amputation, target vessel revascularization (TVR), and disease progression during the follow-up period. Disease progression was defined as the occlusion of the treated vessel, determined by duplex ultrasound or

					angiography, or a > 0.15 decrease in the ankle brachial index of the treated limb. MACE was the composite endpoint composed of stroke, myocardial infarction, and death occurring during the follow-up period.
Lequn et al	Both preoperative and early postoperative hs-CRP level elevations were independent predictors of poor primary patency outcomes. A higher early postoperative hs-CRP level was the only independent inflammatory marker of CD-TLR	Risk Factor	Preoperative	Reintervention and primary patency	Reintervention was defined as clinically driven target lesion revascularization (CD-TLR). Primary patency was defined as freedom

					from CD-TLR and restenosis (determined using a duplex ultrasonography-derived peak systolic velocity ratio of >2.4 and/or >50% stenosis on CTA) at the time of follow-up.
Nardella et al	Elevated IL-1, IL-6, CRP, TNF-α, HMGB-1, OPG and Sortilin levels and low Omentin-1 levels at baseline correlate with worse vascular outcomes in diabetic patients with CLTI.	Risk Factor	Preoperative	MACE e MALE	MACE was defined as composite of myocardial infarction, stroke and cardiovascular death. MALE was defined as composite of acute limb ischemia, major vascular amputations,

					limb-threatening ischemia leading to urgent revascularization
Kumada et al	Preoperative hs-CRP was associated with all-cause mortality, amputation, or both, after lower limb revascularization surgery.	Risk Factor	Preoperative	Amputation, all-cause mortality, or both	Above-ankle amputation of the index limb; death from any cause
Wang et al	Baseline hs-CRP was an independent predictor of restenosis after lower limb angioplasty.	Risk Factor	Preoperative	Restenosis	Restenosis was defined as >50% lumen stenosis or a peak systolic velocity ratio >2.5 at the target lesion on color Doppler ultrasonography.

Legend: EVT: Endovascular Treatment; hsCRP: high sensitivity C reactive protein; IL: Interleukin ; MCP-1: Monocyte chemoattractant protein-1; TNF-α: tumor necrosis factor-alpha; CV: cardiovascular; PAD: Peripheral Artery Disease; ICD: International Classification of Disease; TGF-β: Transforming growth factor-beta; CVAE: cardiovascular adverse events; MACLE: Major adverse cardiovascular limb events; MI :Myocardial Infarction; VCAM-1: vascular cell adhesion molecule 1; ICAM-1: intercellular adhesion molecule 1; OPG: Osteoprotegerin; CLTI: Critical Limb ischemia; MALE: major adverse limb events ; MACE: major adverse cardiovascular event; MMP: matrix metallopeptidase: FM: fat mass; VAS: visceral adiposity score; TLR: target lesion revascularization; TASC: Transatlantic Consensus; ACS: non-fatal acute coronary syndrome; CD-TVR: clinically driven target vessel revascularization ; ABI: ankle-brachial index ; IL1RA: Interleukin 1 Receptor Antagonist; DFU: Diabetic foot ulcers; CTA: Computed Tomography Angiography NA – unavailable data

Table 5. Effect measures and follow-up times.

Author	Marker(s)	Effect measure	Statistical Analysis	Effect measure(s) with [95% CI]	Follow-up Duration
Bleda et al	Hs-CRP	aHR	Cox proportional hazards regression	aHR 1.1 [1.05-1.2]	12 months
Ueki et all	IL-6, TNFα	β	Linear regression	IL-6: 7.51 [3.99-19.77] p=0.951; TNF-α: 0.13 [0.06-0.38] p=0.494	NA
Amrock et al	Hs-CRP	aHR	Cox proportional hazards regression	all-cause mortality: 1.09 [0.94 - 1.27]; CV mortality: 0.90 [0.71 - 1.14]	7.8 years (median)
Mueller et al	Hs-CRP	HR/aHR	Cox proportional hazards regression	Hs-CRP ≥ 5 mg/L: Diabetic <75 years 1.37 [0.85–2.21] p=0.200; Non-diabetic <75 years 2.11 [1.28–3.47] p=0.003; Hs-CRP continuous variable: Diabetic <75 years HR 1.48 [1.02-2.15] p=0.042;	10 years

				Non-diabetic <75 years aHR 2.31 [1.50-3.56] p<0.001	
Schneider et al	TNFR1	HR/aHR	Cox proportional hazard models/ Spearman's correlations / Kaplan–Meier curves	Major amputation: HR 1.37 [1.26–1.48] p<0.0001 ; aHR: 1.29 [1.18–1.42] p<0.0001; Minor amputation: HR 1.29 [1.14–1.46] p<0.0001; aHR 1.11 [0.88–1.41] p=0.396	84 months (median)
Guimarães et al	IL-6, IL-8, IL-10, TNF-α, hs-CRP	NA	Pearsons' chi-quare or Fishers' exact test	*Restenosis-Preprocedural: IL1B: 409,8 ± 64,0; IL-6: 1.846 ± 400,3; IL-8: 302,8 ± 34,5; TNF-a: 155,8 ± 13,8; IL-10: 60,0 ± 10,9; hs-CRP: 2,1 ± 2,5 6 months: IL1B: 335,0 ± 38,7; Il-6: 701,0 ± 102,8; IL-8: 152,3 ± 10,3; TNF-α: 40,5 ± 10,1; IL-10: 127,5 ± 16,6; hs-CRP 0,9 ± 0,8	6 months

				*No restenosis-Preprocedural: IL-1B: 463,1 ± 57,4; IL-6: 1.575,8 ± 290,1; IL-8: 335,2 ± 68,1; TNF-α: 148,2 ± 24,7; IL-10: 57,3 ± 16,1; hs-CRP: 3,7 ± 5,8; 6 months: IL1B: 363, 5 ± 49,1; IL-6: 761,0 ± 116,6; IL-8: 150,4 ± 25,1; TNF-α: 45,3 ± 9,9; IL-10: 119,4 ± 16; hsCRP: 1,10 ± 1,91	
Akkoca et al	Hs-CRP	Mean	Mann-Whitney U test	MACLE: 14.3 ± 20.6 mg/L; non-MACLE: 5.9 ± 10.9 mg/L	5 years
Rief et al	Hs-CRP	aOR	Multivariate logistic regression	MI: 1.005 [1.002–1.009]; Stroke: 1.005 [1.002–1.008]	NA

Sapienza et al (a)	IL-1, IL-6, TNF-α	OR	Logistic regression	IL-6: 1.855 [1.297-2.652] p=0.001; TNF-α: .679 [.512-.902] p=.007; IL1: non-significant	20 ± 9 months (mean)
Gremmels et al	IL-6, IL-8, TNFα	HR	Cox proportional hazards regression	IL-6: 1.58 [1.24–2.0] p = 0.0002; IL-8: 1.93 [1.3–2.9] p = 0.002	39 months
Biscetti et al	IL-6, TNFα, hs-CRP	OR	Logistic regression	**MALE: 1st quartile (Q) – TNF-α: 12.3–28.4; IL-6: 12.2–27.3; hs-CRP: 1.0–3.1; 2nd Q - TNF-α: 28.4–49.8; IL-6: 27.3–38.8; hs-CRP: 3.1–5.3; 3rd Q – TNFa: 50.3–64.4; IL-6: 38.9–45.7; hsCRP: 5.4–7.5; 4th Q - TNF-α: 64.4–74.5; IL-6: 45.7–56.5; hs-CRP: 7.5–10.6	12 months

				(p for trend <0.001; AUROC 1.00 [1.00-1.00]); MACE: 1st Q - TNF-α: 12.3–27.9; IL-6: 12.2–26.4; hsCRP: 1.0–3.1; 2nd Q - TNF-α: 28.4–42.3; IL-6: 26.5–36.5; hs-CRP: 3.1–4.5; 3rd Q - TNF-α: 42.3–63.6; IL-6: 37.1–45.5; hs-CRP: 4.8–7.5; 4th Q - TNF-α: 63.9–74.5; IL-6: 45.6–56.5; hs-CRP: 7.5–10.6; (p for trend <0.001; AUROC 0.91 [0.87-0.94)	
Sapienza et al (b)	IL-1, IL-6, TNF-α	Mean	Pearsons' chi-quare or Fishers' exact test	IL-6 (3 months): occluded graft- 19,93; patent graft-11,07; (6 months): occluded graft- 20; patent graft-10,07; (9 months): occluded graft- 21; patent graft- 8,86; TNF-alfa (3 months): occluded graft-	19.5 months (median)

				19,14; patent graft- 8,99; (6 months): occluded graft- 19,07; patent graft- 8; (9 months): occluded graft- 19,07; patent graft- 7,93; p < 0.0001	
Spychalska nazwolińska et al	TNF-α/Fat Mass ratio; TNF-α/Visceral adipose score ratio	HR	Cox proportional hazard regression and Kaplan-Meier analysis	Reintervention TNF-α/Fat Mass: 0.94 [0.69-1.48] p=0.780; TNF-α/Visceral adipose score: 0.96 [0.64-1.43] p=0.825 MACE TNF-α/Visceral adipose score: Cox's F test p=0.042	12 months
Guo et al	IL-6, Hs-CRP	OR	Logistic regression	IL-6 baseline: 1.11 [1.00- 1,23] p= 0.044; IL-6 24h: 1.04 [1.02-1,06] p<0.001; hs-CRP baseline: 0,57 [0,35-1,80] p= 0,667; hs-CRP 24h 1,15 [1,04-1,26] p= 0,006	6 months
Saenz Pipaon et al	Hs-CRP	aHR	Cox proportional hazards regression	Amputation: 1.70 [1.42-2.05]; MACE: 1.46 [1.28-1.66]	3.6 years (mean)

			(HR corresponds to doubling of hsCRP)		
Bae et al	Hs-CRP	aHR	Cox proportional hazards regression	1.64 [0.80-3.39], log rank p = 0.174	25 months (median)
Fukase et al (d)	Hs-CRP	HR/aHR	Cox proportional hazards regression	all-cause mortality: aHR 2.37 [1.27–4.60] p=0.006; MALE: aHR non significant; HR 1.70 [1.10–2.66] p=0.018	3.5 years (median)
Fukase et al (c)	Hs-CRP	aHR, % (N patients with event/total patients)	Cox proportional hazards regression, log rank	All-cause death: 2.79 [1.66–7.17) p=0.024; MACLE: high hs-CRP vs low hs-CRP 29.0% (23/124) vs 22.1% (32/211) p=0.410; CD-TVR 16.4% (13/124) vs 20.0% (26/211) p=0.682;	3.6 years (median)

				Limb ischemia: 1.0% (1/124) vs 3.0% (4/211) p=0.446; Non-fatal ACS: 1.3% (1/124) vs 0% (0/211) p=0.186; Stroke: 7.3% (5/124) vs 1.6% (2/211) p=0.046; CV death: 2.8% (3/124) vs 0% (0/211) p=0.018;	
Kadoglou et al	Hs-CRP	Mean	T-test	MACE: 10.56 ± 2.92; Non-MACE: 6.33 ± 1.85; p<0.001	6 months
Sega et al	IL1R Antagonist, IL-10	HR	Cox proportional hazards regression	New lesion/recurrence: IL10 : 42.136 [3.799–467.359] p=0.002; IL1RA 1.169 [1.006–1.359] p=0.042	12 months

Kremers et al	IL6	aHR	Cox proportional hazards regression	IL-6: 2.02 [1.35–3.02]	12 months
Caicedo et al	TNF-α	OR	Logistic regression	OR = 2.5 [0.23–136.6] p = 0.0487	12 months
Di et al	Hs-CRP	aHR	Cox proportional hazards regression	MALE: 4.145 [1.628-10.551]; MACE: 1.244 [0.268-5.776]	36 months (median)
Lequn et al	Hs-CRP	aHR	Cox proportional hazards regression	Primary patency: pre-op 2.41 [1.33 - 4.37]; early post-op 1.77 [1.06 - 2.93]; CD-TLR: early post-op 2.37 [1.39 - 4.08] (pre-op non-significant)	21 months (median)
Nardella et al	IL-1, IL-6, TNF-α	Coefficient (Coef)	Logistic regression	MACE IL-1: Coef 0.001 [-0.001-0.004] p=0.362; IL-6: Coef 0.005 [0.002-0.007]	12 months

				p=0.001; TNF-α: Coef 0.001 [-0.002-0.004] p=0.434 MALE IL1: Coef -0.002 [-0.005-0.002] p=0.401; IL6: Coef 0.001 [-0.002-0.005] p= 0.557; TNFα: Coef 0.001 [-0.003-0.004] p= 0.731	
Kumada et al	Hs-CRP	aHR	Cox proportional hazards regression	Amputation: 2.02 [1.02–4.25] p=0.042; Mortality: 1.64 [1.11–2.45] p=0.012; Amputation or mortality: 1.86 [1.30–2.70] p=0.0007	Median 43 months
Wang et al	Hs-CRP	aOR	Logistic regression	1.10 [1.05–1.34] p=0.033	12 months

Legend: aHR – Adjusted hazard Ratio; CI – Confidence Interval; CV – Cardiovascular; HR – Hazard Ratio; aHR – adjusted Hazard Ratio; IL – Interleukin; MACE – Major Adverse Cardiovascular Events; MALE – Major Adverse Limb Events; NA – unavailable data; OR – Odds Ratio;

aOR – adjusted Odds Ratio; ROC- received operating characteristics curve; TNF – Tumor necrosis factor alfa. AUROC – Area Under the Receiving Operating Characteristics Curve. *All measurements are in pg/mL. **Results are presented as ranges per quartile of biomarker concentration.

Supplementary Material

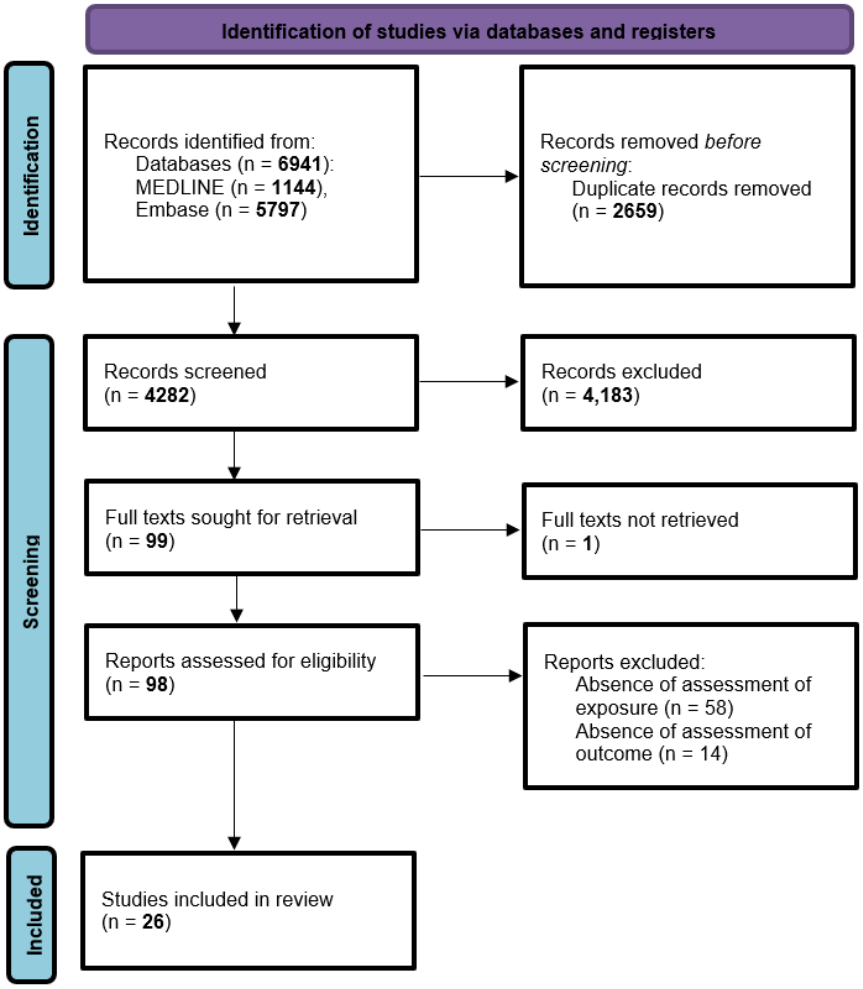

Figure 1S. Study selection process (Prisma flowchart).

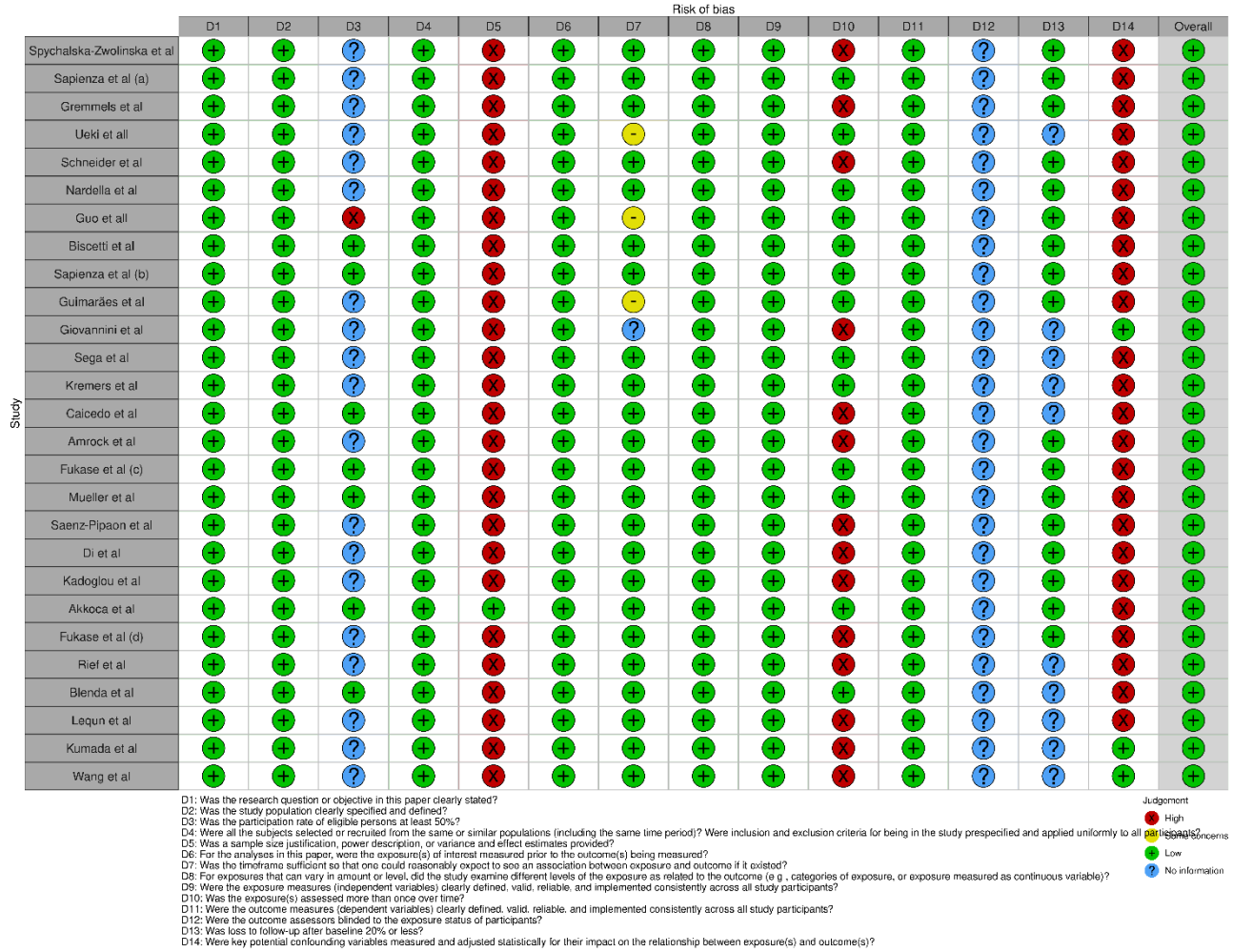

Figure 2S. Risk of bias classification according to prespecified domains.

Table 1S. Search query.

Search terms
((peripheral artery disease[MeSH Terms]) OR (peripheral artery disease) OR (“peripheral arterial disease”) OR (“peripheral vascular disease”) OR (“intermittent claudication”) OR (“claudicants”) OR (“arterial occlusive disease”) OR (“arterial occlusive disease”) OR (“claudicants”) OR (“critical limb ischemia”) OR (“chronic limb-threatening ischemia”) OR (“critical limb-threatening ischemia”)) AND ((c reactive protein[MeSH Terms]) OR ("high-sensitivity c reactive protein") OR (“C-reactive protein”) OR (“high sensitivity C-reactive protein”) OR (“CRP”) OR (“hsCRP”) OR (“ultrasensitive C-reactive protein”) OR (interleukin 1 beta[MeSH Terms]) OR ("interleukin 1 beta") OR (“IL-1β”) OR ("interleukin 18") OR (“IL-18”) OR (interleukin 18[MeSH Terms]) OR (“IL-6”) OR (interleukin 6[MeSH Terms]) OR ("interleukin 6") OR (interleukin 8[MeSH Terms]) OR ("interleukin 8") OR (“IL-8”) OR (“IL-10”) OR (interleukin 10[MeSH Terms]) OR ("interleukin 10") OR (cachectin tumor necrosis factor[MeSH Terms]) OR ("tumor necrosis factor") OR (“TNF”) OR (inducing factor, interferon gamma[MeSH Terms]) OR ("interferon gamma") OR (“IFN”))

Table 2S. Study characteristics.

Author	Journal	Publication Year	Study design	Retrospective (0) or Prospective (1)	Study Center	Country	Continent	Recruitment Time
Bleda et al	Journal of Endovascular Therapy	2015	Cohort	1	Vascular Surgery and Angiology Department, Hospital Universitario de Getafe, Madrid	Spain	Europe	2010 to 2011
Ueki, et all	Sage Journals	2017	Cohort	1	Shinshy University	Japan	Asia	2015 to 2024
Amrock et al	The American Journal of Cardiology	2017	Cohort	1	NHANES	USA	North America	1999 to 2004

Mueller et al	SAGE Open Medicine	2017	Cohort	1	Konventhospital der Barmherzigen Brüder Linz (tertiary care hospital)	Austria	Europe	2000 to 2002
Schneider et al	Cardiovasc Diabetology	2018	Cohort	1	Service de Chirurgie Vasculaire, CHU de Poitiers	France	Europe	2002 to 2012
Guimarães et al	Annals of Vascular Surgery	2018	Cohort	1	Universidade de São Paulo	Brazil	América	NA

Akkoca et al	Anatolian Journal of Cardiology	2018	Cohort	1	Department of General Surgery, University of Health Sciences, Dışkapı Training and Research Hospital; AnkaraNATurkey	Turkey	Europe	NA
Rief et al	Scientific Reports	2018	Cohort	0	Medical University of Graz	Austria	Europe	2005 to 2010
Sapienza et al (a)	International Wound Journal	2019	Cohort	2	University of Rome	Italy	Europe	2010 to 2015
Gremmels et al	Scientific Reporta	2019	Cohort	0	University Medical center Utrecht	the netherlands	Europe	NA
Biscetti et al	Diabetes Care	2019	Cohort	1	Fondazione Policlinico	Italy	Europe	2015 to 2018

					Universitario A. Gemelli IRCCS			
Sapienza et al (b)	International Wound Journal	2019	Cohort	0	University of Rome	Italy	Europe	2012 to 2015
Spychalskanazwolińska et al	International Angiology	2020	Cohort	1	Nicolaus Copernicus University, Bydgoszcz	Poland	Europe	NA
Guo et al	Sage Journals	2020	Cohort	1	Changai Hospital	China	Asia	2013 to 2014
Saenz Pipaon et al	Journal of Extracellular Vesicles	2020	Cohort	1	Department of Vascular Surgery, Complejo Hospitalario de Navarra	Spain	Europe	2010 to 2018
Bae et al	Journal of Thrombosis and Thrombolysis	2020	Cohort	1	Gyeongsang National University Hospital	South Korea	Asia	2012 to 2015

Fukase et al (d)	Journal of Cardiology	2020	Cohort	1	Juntendo University Hospital (Tokyo, Japan)	Japan	Asia	2009 to 2018
Fukase et al (c)	Heart and vessels	2021	Cohort	0	Department of Cardiovascular Biology and Medicine, Juntendo University Graduate School of Medicine	Japan	Asia	2009 to 2020
Kadoglou et al	Cardiovascular Diabetology	2021	Cohort	1	NA	Greece	Europe	NA
Sega et al	International Journal of Molecular Sciences	2022	Cohort	1	Diabetic Foot Unit of the Maria Cecilia Hospital (Cotignola, Italy)	Italy	Europe	2018 to NA

Kremers et al	Scientific Reporta	2022	Cohort	1	department of Vascular Surgery of the Maastricht University Medical Center (MUMC+)	Netherlands	Europe	2018 to 2020
Caicedo et al	biomedicines	2022	Cohort	1	Angiology and Vascular Surgery Department of the Clinical Hospital of Pontevedra, Spain	Spain	Europe	2016 to 2018
Di et al	Annals of Vascular Surgery	2022	Cohort	1	Peking Union Medical College Hospital	China	Asia	2014 to 2017
Lequn et al	Annals of Vascular Surgery	2022	Cohort	0	National Center for Cardiovascular Disease, Beijing	China	Asia	2017 to 2020

					Fuwai Hospital, Vascular Surgery Center			
Nardella et al	Cardiovascular Diabetology	2023	Cohort	1	Fondazione Policlinico Universitario A. Gemelli Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS)	Italy	Europe	2019 to 2022
Kumada et al	Journal of Clinical Medicine	2023	Cohort	0	Matsunami General Hospital and Nagoya Kyoritsu Hospital	Japan	Asia	2009 to 2018
Wang et al	Frontiers in Cardiovascular Medicine	2022	Cohort	0	People's Liberation Army Strategic Support Force	China	Asia	2016 to 2020

					Characteristic Medical Center			

Table 3S. Biomarker biological sample and method in included studies.

Author	**Type of sample (0- serum, 1- plasma, 2- whole blood)**	**Method**	**Analyzer**	**Biomarker (Yes/No and cutoff)**
Bleda et al	NA	Immunoassay	Roche Diagnostics, Indianapolis, In, USA	No
Ueki et all	2	IL-6: chemiluminescent enzyme imunossay kits; TNF-a: specific enzyme-linked imunosobernt assay kits	Colin Wave-form analyzer (BP-203RPE; Omron Colin, Tokyo, Japan)	No
Amrock et al	0	NA	Biochemistry automatic analyzer (H7180; Hitachi High-Tech Co., Ltd., Tokyo, Japan)	No

Mueller et al	0	C-reactive protein (CRP) was measured by a high-sensitivity assay (N High Sensitivity CRP)	BN ProSpec analyzer (Dade Behring)	Yes and no: Hs-CRP ≥ 5.0 mg/L (vs <5.0 mg/L) and continuous
Schneider et al	0	TNFR1 ELISA kit (EKF Diagnostics, Dublin, Ireland)	ADAMS A1c HA-8160 analyser; Menarini, Florence, Italy	No
Guimarães et al	2	Commercial assay kit (IMMULITE; Diagnostic Products, Los Angeles, CA, USA) with a solid-phase, two-site chemiluminescent immunometric assay	NA	No
Akkoca et al	1	NA	NA	No

Rief et al	NA	NA	NA	No
Sapienza et al (a)	1 and 2	Quantikine enzyme-linked immunosorbent assay (ELISA) kits	NA	No
*Gremmels et al**	NA	A multiple cytokine assay (Bio-rad, Hercules, CA)	NA	No
Biscetti et al		Quantikine ELISA Kit (R&D Systems, Minneapolis, MN)	NA	No
Sapienza et al (b)	1 and 2	Quantikine ELISA kits (R&D Systems, Inc., Minneapolis, MN).	BCS XP analyser (Siemens Healthcare Diagnostics, Tarrytown NY)	No
Spychalska nazwolińska et al	2	ELISA kit from CUSABIO (cat. no. CSB-E04740h)	(TANITA BC-420 MA device, Tanita Corporation, Tokyo, Japan)	Yes (TNF-α concentration indexed to FM that was greater than or equal

				to 1.57 or a TNF-α -to-VAS ratio greater than or equal to 2.76)
Guo et al	0 and 2	IL-6: enzyme-linked immunosorbent assay; Hs-CRP: rate neplhelometry	NA	No
Saenz Pipaon et al	0	immunoassay	Immulite; Diagnostic Product Corporation	No
Bae et al	NA	Enzyme-linked immunosorbent assay	UniCel® DxC 800 Synchron® Clinical System (Beckman Coulter, Inc., Brea, CA, USA)	Yes (≥3 mg/dl)
Fukase et al (d)	-	NA	NA	Yes (≥ 0.15 mg/dl)
Fukase et al (c)	1	Kit (LZ test, EIKEN CRP-HG; Eiken Chemical Co., Ltd., Tokyo, Japan) by the latex agglutination method		Yes; Hs-CRP (dichotomized to > or ≤ 0.15 mg/dl) using receiver-operating characteristic (ROC) analysis

Kadoglou et al	0	Latex-enhanced immunonephelometry	(Dade Behring, Marburg, Germany)	No
Sega et al	0	Commercial ELISA kit (Thermofisher, Waltham, MA, USA)	NA	No
Kremers et al	1	ProSeek Cardiovascular II and III panels (Olink Biosciences, Uppsala, Sweden)	NA	No
Caicedo et al	1	Concentration micro kit (K0842, Thermo Fisher, Vilnius, Lithuania).	NA	Yes (TNF≥ 8.1; hs-CRP>0.5 mg/dL)
Di et al	NA	Immunoturbidimetry assay	BECKMAN AU analyzer (Beckman Coulter, United States) with a detection range of 0.08 mg/L to 160 mg/L	Yes (>3 mg/dl)
Lequn et al	NA	NA	NA	No

Nardella et al	2	ELISA kits (MBS012415 for IL-1, EH0201 for IL-6, EH0302 for TNF-α)	NA	No
Kumada et al	0	Latex-enhanced, highly sensitive CRP immunoassay	NA	Yes (>12.6 mg/L)
Wang et al	NA	NA	NA	No

Legend: Hs-CRP – High-sensitivity C reactive protein; IL – Interleukin; NA – unavailable data; TNF-α – Tumor Necrosis Factor α, FM: fat mass, VAS: visceral adiposity score.

Table 4S. Covariates used in the adjusted models.

Author	Covariates used in adjusted models
Spychalska-Zwolińska et al	Age (years); Smoking habit; Diabetes mellitus; LDL cholesterol (mg/dl); Type of femoropopliteal lesion according to TASC-II; Length of stent (mm); Leptin-to-TNF-α ratio; TNF-α -to-FM (kg) ratio; TNF-α -to-VAS ratio.
Sapienza et al (a)	Vessel run-off; VCAM-1; ICAM-1; IL-6; TNF-α
Gremmels et al	Age; Sex; GFR; Presence of Diabetes; Hb; ABI; Leriche-Fontaine Classification; CRP
Schneider et al	Sex (ref. women); SBP (mmHg); DBP (mmHg); eGFR; uACR; Severe diabetic retinopathy; Macular edema; History of PAD; TNFR1; ANGPTL2
Nardella et al	Male sex; Age; Diabetes duration; BMI; Smoking (current); Smoking (former); Hypertension; Hypercholesterolemia; CAD; CVD; Rutherford II-4; Rutherford III-5; Rutherford III-6; HbA1c; FBG; Total cholesterol; LDL; HDL; Triglycerides; Creatinine; IL1; IL6; CRP; TNFα; OPG; HMGB1; Omentin1; Sortilin; Constant
Guo et al	Smoking; Diabetes mellitus; Lesion length; Cumulative stent length; Number of stents
Biscetti et al	Age; Sex; BMI; High Blood Pressure; Diabetes Duration; Smoking Status (Current; Former; Never); Rutherford Staging; Previous Cardiovascular and Cerebrovascular Events; Treatment (Oral Antidiabetic Agents; insulin); Total Cholesterol; LDL Cholesterol; Triglycerides; FBG; Hba1c
Kremers et al	Age; Gender; Prior myocardial infarction; Stroke; Creatinine levels
Amrok et al	Statins; Angiotensin-converting enzyme inhibitors or angiotensin II receptor blocker medications; Antihypertensives; Antiplatelet agents
Fukase et al (a)	Age; Sex; Hemodialysis; Statin use; Cilostazol use; Ejection fraction ; Higher hs-CRP; CTLI; TASC II classification C/D; Chronic total occlusion lesion; Below-knee artery lesion
Mueller et al	Patient age ≥65 years; Arterial hypertension; Cardiovascular comorbidity; Critical limb ischaemia; ABI< 0.60 mmHg/mmHg; History of a PAD-specific intervention; eGFR< 60 mL/min/1.73 m2; Hs-CRP ≥5.0 mg/L; NT-proBNP ≥125 ng/L
Saenz Pipaon et al	Age; Sex; Diabetes mellitus; Hypertension; Dyslipidaemia; Estimated glomerular filtration rate (eGFR); Hs-CRP
Di et al	Uric acid; Hs-CRP; Free fatty acid; Diabetes melitus; Chronic obstructive pulmonary disease
Fukase et al (b)	LDL-C level; HbA1c level; Lower pre-treatment ABI (< 0.69; as the median); Presence of a femoropopliteal artery lesion; Presence of chronic total occlusion (CTO) lesion; Aspirin
Rief et al	Age (years); Hs-CRP (mg/L); Diabetes melitus; MPV (fl); Thrombocytosis
Bleda et al	Age (cutoff point 68 years); Diabetes mellitus; Hypertension; Dyslipidemia; Smoking history; Coronary heart disease; Cerebrovascular disease; Renal disease
Lequn et al	NLR and Hs-CRP level (high; moderate; and low); Age; Sex; Chronic total occlusion; Rutherford classification; Lesion length; Severe calcification; TASC II classification; Tibial runoff; Type 2 diabetes; Coronary heart disease; History of stroke; and Hyperlipidemia.

Kumada et al	male sex, age, previous coronary artery disease, endovascular therapy (vs. bypass surgery), below-knee artery disease, and ulcer/gangrene as covariates with $p < 0.05$ in a univariate analysis
Wang et al	NA

Legend: TNF-α: tumor necrosis factor-alpha; FM: fat mass; VAS: visceral adiposity score; VCAM-1: vascular cell adhesion molecule 1; ICAM-1: intercellular adhesion molecule 1; IL: Interleukin ; eGFR – Glomerular Filtration Rate; CRP – C reactive protein; SBP- Systolic Blood Pressure; DBP- Diastolic Blood Pressure; uACR: Urine Albumin-Creatinine Ratio; PAD – Peripheral Artery Disease; BMI – Body Mass Index; CAD- Coronary artery disease; CVD- Cardiovascular disease; FBG- Fasting Blood Glucose; OPG- Osteoprotegerin; HMGB1- High-Mobility Group Box-1; LDL– Low Density Lipoprotein; HDL- High Density Lipoprotein; CTLI- Critical Limb ischemia; ABI – Ankle Brachial Index; MPV- Mean Platelet Volume; NLR – Neutrophile-to-Lymphocyte ratio; TASC – Transatlantic Consensus.

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Página 1, Parágrafo 1: “Association of Inflammatory Biomarkers with Morbidity and Mortality Risk in Patients with Peripheral Artery Disease: A Systematic Review and Meta-Analysis”
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Página 4, Parágrafo 4: “PAD affects over 27 million people in Europe and North America [3] and is a major contributor to limb loss and death worldwide [4] … Research has shown that the development of atherosclerosis is driven by an inflammatory process [6-8]… Therefore, there is interest in using blood biomarkers to monitor prognosis and treatment efficacy in PAD patients.”
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Página 7, Parágrafo 2:” This systematic review and meta-analysis aims to assess the association between selected inflammatory biomarkers and morbidity and mortality risk in PAD patients.”
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Página 7, Parágrafo 6:” Included studies were prospective cohorts, retrospective cohorts, case-control studies, or post hoc analyses of randomized controlled studies. Other inclusion criteria comprised 1) patients aged 18 years or older; 2) with a diagnosis of peripheral artery disease; 3) a minimum of 1 of the pre-specified biomarkers measured in the peripheral blood previous to the occurrence of the outcomes of interest; 4) describe at least one of the outcomes of interest (all-cause mortality, cardiovascular mortality, stroke, myocardial infarction, congestive heart failure, major adverse cardiovascular events - MACE, untreated loss of patency of a revascularization procedure, re-intervention on a revascularization procedure or recurrence of disease, major amputation, major adverse limb events – MALE, major adverse cardiovascular and limb events - MACLE); 5) describe the association of at least one measured biomarker with one outcome of interest. The pre-specified biomarkers included: high-sensitivity (hs) CRP, TNF-alpha, interferon-gamma inducing factor, IL-1β, IL-6, IL-8, IL-10, and IL-18. Exclusion criteria encompassed 1) studies with less than 20 patients; 2) studies published in abstract form; 3) studies not published in the English language; 4) studies published before 1/January/2014; 5) duplicate manuscripts; and 6) case-reports and case series..”
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Página 7, Parágrafo 4:” A MEDLINE and EMBASE database search was performed to find evidence up to the 28th of March 2024 on the association between inflammatory biomarkers and morbidity and mortality risk in PAD patients (Supplemental Table 1S).”
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Página 7, Parágrafo 4:” A MEDLINE and EMBASE database search was performed to find evidence up to the 28th of March 2024 on the association between inflammatory biomarkers and morbidity and mortality risk in PAD patients (Supplemental Table 1S).”
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they	Página 7, Parágrafo 5:” Two reviewers independently screened search records for inclusion (FM, MFM), and another checked decisions (JRN). Titles and abstracts were screened at this stage and relevant studies were selected for full-text analysis…. Two

Section and Topic	Item #	Checklist item	Location where item is reported
		worked independently, and if applicable, details of automation tools used in the process.	reviewers (MFM, FM) blindly and independently checked the full texts, decided reported when existent. on the inclusion of individual studies… A senior reviewer decided on disagreements between reviewers (JRN).”
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Página 8, Parágrafo 3:” Two reviewers (MFM, FM) blindly and independently checked the full texts, decided reported when existent. on the inclusion of individual studies, and extracted data about study characteristics, inflammatory biomarkers, and events. A senior reviewer decided on disagreements between reviewers (JRN).”
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Página 8, Parágrafo 3:” Extracted data included effect measures or descriptives of the pre-specified included outcomes, presented as Hazard Ratios (HR), Odds Ratios (HR), or mean and standard deviation. Ninety-five percent Confidence Intervals (CI) were reported when existent.”
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Página 8, Parágrafo 3:” Extracted data included effect measures or descriptives of the pre-specified included outcomes, presented as Hazard Ratios (HR), Odds Ratios (HR), or mean and standard deviation. Ninety-five percent Confidence Intervals (CI) were reported when existent.”
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Página 8, Parágrafo 4:” Concerning qualitative assessment, the National Heart, Lung, and Blood Institute (NHLB) Quality Assessment Tool for Observational Cohort and Cross Sectional Studies was used to assess the risk of bias, adequate reporting and quality of statistical analysis of observational cohorts and cross-sectional studies. Evaluated bias domains included bias related to the research question, study population, sample size, exposure, outcomes, loss to follow-up, and confounding variables.”
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Página 8, Parágrafo 3:” Extracted data included effect measures or descriptives of the pre-specified included outcomes, presented as Hazard Ratios (HR), Odds Ratios (HR), or mean and standard deviation.”
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Página 8, Parágrafo 3:” Two reviewers (MFM, FM) blindly and independently checked the full texts, decided reported when existent. on the inclusion of individual studies, and extracted data about study characteristics, inflammatory biomarkers, and events. A senior reviewer decided on disagreements between reviewers (JRN).”
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Página 8, Parágrafo 3:” Two reviewers (MFM, FM) blindly and independently checked the full texts, decided reported when existent. on the inclusion of individual studies, and extracted data about study characteristics, inflammatory biomarkers, and events. A senior reviewer decided on disagreements between reviewers (JRN). Extracted data included effect measures or descriptives of the pre-specified included outcomes, presented as Hazard Ratios (HR), Odds Ratios (HR), or mean and standard deviation. Ninety-five percent Confidence Intervals (CI) were reported when existent.”
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Página 9, Parágrafo 1:” Assessment of reporting bias was performed with the Egger’s regression test and respective funnel plot. Heterogeneity was evaluated through Q statistic-related measures of variance (Tau2, I2, Chi-squared test), and a random-effects model with an inverse variance method was preferred to compute estimates for the summary effect. Forest plots were used to display results from the meta-analysis, where the measure of effect for each study is represented by a square and the

Section and Topic	Item #	Checklist item	Location where item is reported
			respective areas are proportional to study weight. A p value inferior to 0.05 was considered statistically significant for all analyses.
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Página 9, Parágrafo 1:" …a random-effects model with an inverse variance method was preferred to compute estimates for the summary effect. Forest plots were used to display results from the meta-analysis, where the measure of effect for each study is represented by a square and the respective areas are proportional to study weight. A p value inferior to 0.05 was considered statistically significant for all analyses. Computations were conducted using Review Manager 5.3 and Stata 16.1."
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Página 9, Parágrafo 1:" Heterogeneity was evaluated through Q statistic-related measures of variance (Tau2, I2, Chi-squared test), and a random-effects model with an inverse variance method was preferred to compute estimates for the summary effect.
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Página 9, Parágrafo 1:" Heterogeneity was evaluated through Q statistic-related measures of variance (Tau2, I2, Chi-squared test), and a random-effects model with an inverse variance method was preferred to compute estimates for the summary effect. Forest plots were used to display results from the meta-analysis, where the measure of effect for each study is represented by a square and the respective areas are proportional to study weight."
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Página 9, Parágrafo 1:" Assessment of reporting bias was performed with the Egger's regression test and respective funnel plot."
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Página 9, Parágrafo 1:" Heterogeneity was evaluated through Q statistic-related measures of variance (Tau2, I2, Chi-squared test), and a random-effects model with an inverse variance method was preferred to compute estimates for the summary effect. Forest plots were used to display results from the meta-analysis, where the measure of effect for each study is represented by a square and the respective areas are proportional to study weight."
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Página 9, Parágrafo 3:" A total of 6941 records were identified through database searching, of which 4282 were kept after duplicates were removed (Supplemental Figure 1S). Ninety-eight publications were assessed for eligibility through their full texts, with 26 manuscripts selected for qualitative synthesis. Due to heterogeneity between studies, only manuscripts with hs-CRP as exposure were included in the quantitative synthesis."
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Página97, Parágrafo 3:" Of those, three publications were excluded from the analysis since the outcome of interest was compared without using a longitudinal effect measure. One additional study was not included in the quantitative synthesis as it presented myocardial infarction and stroke as outcomes, which were not evaluated in any other studies with hs-CRP as exposure. When more than one estimate was presented in a given study, the adjusted estimates for hs-CRP as a continuous variable were preferred for quantitative synthesis. Supplemental figure 1S depicts the study selection process."
Study characteristics	17	Cite each included study and present its characteristics.	Página 10, Parágrafo 1:" Included research papers were published between 2017 and 2023, with a wide range of locations (US, Brazil, Japan, China, South Korea, and several countries in Europe). All papers were observational and based on a prospective or retrospective cohort (Supplemental Table 2S), with one of the included studies being

Section and Topic	Item #	Checklist item	Location where item is reported
			a post hoc of a randomized controlled trial. The total number of included patients with PAD for quantitative synthesis was 2283 (all-cause mortality), 1738 (MACE), 356 (MALE), and 296 (revascularization)…. Diabetes prevalence represented 25.1% to 100% of each cohort's patients (Table 1). Selected research papers included both surgical and non-surgical settings, with the latter including both outpatient and inpatient contexts (Table 2). Rutherford's class variedacross included studies, although most patients did not present prior PAD revascularization (Table 3)."
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Página 11, Parágrafo 2:" The risk of bias is represented in Fig. 2S (supplementary materials). All included studies, with the exception of Akkoca et al, did not present sample size justification or power description, although effect estimates were provided in almost all studies – for this reason, domain 5 of the NHLB tool was classified as high-risk of bias for most studies. Information was lacking in most research papers on the blinding of outcome assessors, as well as on the participation rate of eligible participants. Some included papers used adjusted time-dependent analysis, and thus were considered as having low risk in domain 14, even though sensitivity or competing risks analyses (e.g., Fine and Gray's model) were not performed for competing risks (Figure 2S). Overall, all selected studies were considered as having a low risk of bias as per the NHLB tool. Tables 4 and 5 present, for each study, outcome definitions, effect measures, and follow-up times"
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Página 10, Parágrafo 1:" The majority of included studies presented as prognostic markers TNF-α, IL-6, and hs-CRP. Quantification of hs-CRP was based on immunoassays, although the specific assay varied across studies, with some not providing information on the method or analyzer (Supplemental Table 3). Of the studies included in quantitative synthesis, MACE was defined as 1) composite endpoint of stroke, myocardial infarction, and death [36], and 2) CV death, non-fatal MI, and non fatal stroke [37]. Revascularization in the meta-analysed studies was defined as 1) [40]. reintervention within 12 months of endovascular treatment [38]; and 2) clinically driven target lesion revascularization [39]. Finally, MALE was defined in the studies selected for quantitative analysis as 1) a composite endpoint of limb amputation, target vessel revascularization, and disease progression [36]; 2) a composite of clinically driven target vessel revascularization and target limb major amputation, and all-cause death[40]. … Diabetes prevalence represented 25.1% to 100% of each cohort's patients (Table 1). Selected research papers included both surgical and non-surgical settings, with the latter including both outpatient and inpatient contexts (Table 2). Rutherford's class variedacross included studies, although most patients did not present prior PAD revascularization (Table 3)."
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Página 11, Parágrafo 2:" All included studies, with the exception of Akkoca et al, did not present sample size justification or power description, although effect estimates were provided in almost all studies – for this reason, domain 5 of the NHLB tool was classified as high-risk of bias for most studies. Information was lacking in most research papers on the blinding of outcome assessors, as well as on the participation rate of eligible participants. Some included papers used adjusted time-dependent analysis, and thus were considered as having low risk in domain 14, even though sensitivity or competing risks analyses (e.g., Fine and Gray's model) were not performed for competing risks (Figure 2S). Overall, all selected studies were considered as having a low risk of bias as per the NHLB tool. Tables 4 and 5 present,

Section and Topic	Item #	Checklist item	Location where item is reported
			for each study, outcome definitions, effect measures, and follow-up times."
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Página 11, Parágrafo 3:" IL-6 Loss of Patency/Restenosis Loss of patency or restenosis was evaluated for IL-6 in 4 of the studies included for qualitative synthesis [11,41-44]. All of these studies had pre and postoperative samples in which IL-6 was quantified and were performed in a surgical context where the type of surgery was either lower limb angioplasty [11,41-44], lower limb bypass and lower limb angioplasty [38], or midfoot amputation and lower limb angioplasty [43]. Follow up varied between 6 and 22 months and a higher level of pre/postoperative IL-6 was identified as a risk factor for loss of patency in 3 studies [42-44]. Conversely, 2 studies did not suggest an association of pre/postoperative IL-6 levels with restenosis [11,41]. "a Página 16, Parágrafo 2 "Revascularization Revascularization was reported in 2 studies [38,39]. A total of 296 patients were included in the quantitative analysis and median follow-up time ranged between 12 and 21 months. Covariate adjustment is described in Table 4S. The pooled estimate was HR 1.54 [0.73–3.25] (p =0.26; I2 = 87%) and the respective forest plot is represented in Figure 5. Figure 5. Association between hs-CRP and revascularization (forest plot). "
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Página 11, Parágrafo 2:" All included studies, with the exception of Akkoca et al, did not present sample size justification or power description, although effect estimates were provided in almost all studies – for this reason, domain 5 of the NHLB tool was classified as high-risk of bias for most studies. Information was lacking in most research papers on the blinding of outcome assessors, as well as on the participation rate of eligible participants. Some included papers used adjusted time-dependent analysis, and thus were considered as having low risk in domain 14, even though sensitivity or competing risks analyses (e.g., Fine and Gray's model) were not performed for competing risks (Figure 2S). Overall, all selected studies were considered as having a low risk of bias as per the NHLB tool. Tables 4 and 5 present, for each study, outcome definitions, effect measures, and follow-up times."
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Página 11, Parágrafo 2:" All included studies, with the exception of Akkoca et al, did not present sample size justification or power description, although effect estimates were provided in almost all studies – for this reason, domain 5 of the NHLB tool was classified as high-risk of bias for most studies. Information was lacking in most research papers on the blinding of outcome assessors, as well as on the participation rate of eligible participants. Some included papers used adjusted time-dependent analysis, and thus were considered as having low risk in domain 14, even though sensitivity or competing risks analyses (e.g., Fine and Gray's model) were not performed for competing risks (Figure 2S). Overall, all selected studies were considered as having a low risk of bias as per the NHLB tool. Tables 4 and 5 present, for each study, outcome definitions, effect measures, and follow-up times."
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Página 15, Parágrafo 1:" Publication bias for this outcome was evaluated by a funnel plot and Egger's regression test (p = 0.0002), which suggests presence of bias – Figure 2. Figure 1. Association between hs-CRP and all-cause mortality (forest plot). Figure 2. Funnel plot depicting absence of publication bias for all-cause mortality and hs-CRP."
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Página 11, Parágrafo 2:" All included studies, with the exception of Akkoca et al, did not present sample size justification or power description, although effect estimates were provided in almost all studies – for this reason, domain 5 of the NHLB tool was

Section and Topic	Item #	Checklist item	Location where item is reported
			classified as high-risk of bias for most studies. Information was lacking in most research papers on the blinding of outcome assessors, as well as on the participation rate of eligible participants. Some included papers used adjusted time-dependent analysis, and thus were considered as having low risk in domain 14, even though sensitivity or competing risks analyses (e.g., Fine and Gray's model) were not performed for competing risks (Figure 2S). Overall, all selected studies were considered as having a low risk of bias as per the NHLB tool. Tables 4 and 5 present, for each study, outcome definitions, effect measures, and follow-up times."
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Página 16, Parágrafo 3:" The study's overall findings demonstrated the predictive power of inflammatory biomarkers for clinical outcomes in patients with PAD, with variations in the strength of these associations depending on the specific biomarker and clinical context…. The identified biomarkers, including hs-CRP, IL-6, TNF-α, IL-1, IL-10, and IL-8, are known mediators of inflammation and are implicated in the pathogenesis of atherosclerosis and its complications, which underlies PAD [62]. Elevated levels of these biomarkers reflect the inflammatory response within atherosclerotic plaques, promoting plaque instability, endothelial dysfunction, and thrombosis….The correlations observed between inflammatory biomarkers and adverse clinical outcomes in peripheral arterial disease (PAD) patients in this manuscript may have clinical implications for treatment and prognosis. According to these results, inflammatory biomarkers may be useful instruments for risk stratification, allowing medical professionals to identify high-risk patients who are more likely to experience complications including MACE, MALE, loss of patency, or death. These patients might benefit from tailored interventions meant to reduce these risks and enhance patient conditions."
	23b	Discuss any limitations of the evidence included in the review.	Página 21, Parágrafo 1:" However, this study has limitations. First, the heterogeneity of the included studies, including differences in study design, patient populations, and interventions, may introduce variability in the results and limit the ability to draw broad conclusions. Second, variations in biomarker measurement methods across studies based on immunoassays with differing methods and analyzers could affect the accuracy and comparability of the findings. Third, potential confounding factors may not have been fully accounted for in the analyses, leading to biased results. Additionally, publication bias was identified for the association of hs-CRP with all-cause mortality, indicating the presented results might not be the full picture. All included studies had a mean age above 60 years, which may limit the applicability of the findings to younger populations. Information was also lacking in most studies regarding the blinding of outcome assessors and the participation rate of eligible participants, which could introduce biases in outcome assessment and impact the validity of the results. Finally, the generalizability of the findings may be influenced by the specific patient populations and clinical contexts represented in the included studies, reducing their applicability to broader or different settings"
	23c	Discuss any limitations of the review processes used.	Página 21, Parágrafo 1:" However, this study has limitations. First, the heterogeneity of the included studies, including differences in study design, patient populations, and interventions, may introduce variability in the results and limit the ability to draw broad conclusions. Second, variations in biomarker measurement methods across studies based on immunoassays with differing methods and analyzers could affect the

Section and Topic	Item #	Checklist item	Location where item is reported
			accuracy and comparability of the findings. Third, potential confounding factors may not have been fully accounted for in the analyses, leading to biased results. Additionally, publication bias was identified for the association of hs-CRP with all-cause mortality, indicating the presented results might not be the full picture. All included studies had a mean age above 60 years, which may limit the applicability of the findings to younger populations. Information was also lacking in most studies regarding the blinding of outcome assessors and the participation rate of eligible participants, which could introduce biases in outcome assessment and impact the validity of the results. Finally, the generalizability of the findings may be influenced by the specific patient populations and clinical contexts represented in the included studies, reducing their applicability to broader or different settings"
	23d	Discuss implications of the results for practice, policy, and future research.	Página 22, Parágrafo 1:" In conclusion, the study provides valuable insights into the associations between inflammatory biomarkers and adverse clinical outcomes in PAD patients. These findings highlight the intricate link between inflammation and the pathophysiology of PAD, emphasizing the potential of inflammatory biomarkers as prognostic indicators. Moving forward, further research is warranted to validate these associations, elucidate underlying mechanisms, and explore the clinical utility of inflammatory biomarkers in guiding personalized management strategies for PAD patients … The study highlights the ability of inflammatory biomarkers to predict clinical outcomes in PAD patients. The strength of these associations varies based on the specific biomarker and clinical context"
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Página 7, Parágrafo 3:" The study protocol was registered in PROSPERO (PROSPERO ID: CRD42024530001) per standard reporting conventions."
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Página 7, Parágrafo 3:" The study protocol was registered in PROSPERO (PROSPERO ID: CRD42024530001) per standard reporting conventions."
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Página 7, Parágrafo 3:" The study protocol was registered in PROSPERO (PROSPERO ID: CRD42024530001) per standard reporting conventions."
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Página 22, Parágrafo 3:" The authors declare that no funding was received for conducting this research."
Competing interests	26	Declare any competing interests of review authors.	Página 22, Parágrafo 4:" The authors report no conflict of interest."
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Página 7, Parágrafo 3:" The study protocol was registered in PROSPERO (PROSPERO ID: CRD42024530001) per standard reporting conventions."

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Section and Topic	Item #	Checklist item	Reported (Yes/No)
TITLE			
Title	1	Identify the report as a systematic review.	Yes
BACKGROUND			
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	Yes
METHODS			
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.	Yes
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	Yes
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.	Yes
Synthesis of results	6	Specify the methods used to present and synthesise results.	Yes
RESULTS			
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	Yes
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).	Yes
DISCUSSION			
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	Yes
Interpretation	10	Provide a general interpretation of the results and important implications.	Yes
OTHER			
Funding	11	Specify the primary source of funding for the review.	Yes
Registration	12	Provide the register name and registration number.	Yes

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71.

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