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Unraveling the Mechanisms of Neutrophil Extracellular Trap Formation and the Role of Reactive Oxygen Species in Myocardial Bridging

ABSTRACT

Myocardial bridging (MB) is a congenital coronary anomaly in which a segment of a coronary artery courses through the myocardium, resulting in systolic compression and alterations in coronary vasoreactivity. Consequently, morphological, functional, and hemodynamic changes may occur, with various clinical implications. Although traditionally considered a benign anatomical variant, accumulating evidence suggests an association between MB and endothelial dysfunction, oxidative stress, local inflammatory activation, and atherosclerotic plaque formation, predominantly in the arterial segment proximal to the bridge. Reduced wall shear stress in the proximal segment is associated with a pro-inflammatory and pro-oxidative vascular environment characterized by reduced nitric oxide (NO) bioavailability, despite increased endothelial nitric oxide synthase (eNOS) expression, consistent with impaired eNOS signaling and possible functional uncoupling, together with activation of reactive oxygen species-generating pathways. Myeloperoxidase (MPO), released from activated neutrophils, is a key mediator of oxidative vascular injury and may contribute to endothelial dysfunction by reducing NO bioavailability, generating highly reactive oxidants such as hypochlorous acid, and promoting oxidative endothelial injury. Hemodynamic alterations characteristic of MB may promote MPO activation and the MPO–H_2O_2 pathway, thereby facilitating mechanisms involved in neutrophil extracellular trap (NET) formation and contributing to the pro-inflammatory vascular environment. MPO- and NET-mediated pathways may represent biologically plausible contributors to endothelial dysfunction and proximal atherogenesis in MB. However, the role of these pathways in the pathophysiology of MB remains insufficiently investigated and should currently be regarded as a hypothesis rather than an established pathophysiological mechanism.

Keywords: Endothelial dysfunction, myeloperoxidase, myocardial bridging, neutrophil extracellular traps, oxidative stress, reactive oxygen species, shear stress

REVIEW

INTRODUCTION

The uniqueness of the coronary circulation lies in its crucial role in generating arterial pressure necessary for the perfusion of the systemic circulation. Myocardial bridging (MB), in which a segment of a coronary artery courses intramurally through the myocardium, additionally causes compression of the tunneled coronary segment, thereby impairing coronary perfusion during the systolic phase of the cardiac cycle.

Morphological and hemodynamic disturbances of coronary blood flow associated with MB, as well as the imbalance between myocardial oxygen supply and demand, may be associated with angina pectoris, myocardial ischemia, acute coronary syndrome, left ventricular dysfunction, arrhythmias, and even sudden cardiac death.[1]

Therefore, the regulation and control of coronary blood flow, as well as the factors influencing myocardial oxygen consumption, are essential for understanding the pathophysiological basis of MB, which is characterized by oxidative stress, endothelial dysfunction, inflammation,[1,2] and potentially NETosis.

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Endothelial dysfunction occurs through the rapid scavenging of nitric oxide (NO) by superoxide anions generated in response to oxidative stress. Oxidative stress disrupts the synthesis and bioavailability of NO, further impairing endothelial function and promoting vasoconstriction, thrombosis, and inflammation.[3,4]

The major endothelium-dependent biochemical pathways rely on the presence of a functional endothelium, particularly on the endothelium-derived relaxing factor identified as NO.[5] Nitric oxide is synthesized and released by vascular endothelial cells through the enzymatic conversion of L-arginine to citrulline mediated by endothelial NO synthase (eNOS). It inhibits the development of coronary atherosclerosis and promotes endothelial repair by facilitating potassium channel opening, vasodilation, and scavenging reactive oxygen species (ROS).[6]

Nitric oxide–mediated vasodilation is impaired in MB and results not only from endothelial dysfunction but also from the transformation of the vascular surface into a pro-inflammatory and pro-thrombotic monolayer. Previous studies have shown that the proximal segment of MB is characterized by reduced NO bioavailability, increased oxidative stress, endothelial dysfunction, and a greater susceptibility to the development of atherosclerotic lesions.[2,7]

Studies have demonstrated that, compared with normal coronary arteries, inflammation and the neutrophil-to-lymphocyte ratio are significantly increased in patients with MB.[1,8] Under conditions of infiltration and inflammation, neutrophils release neutrophil extracellular traps (NETs) as a defense mechanism.

NETosis represents a key neutrophil-mediated immune response mechanism involving robust generation of ROS through NADPH oxidase activity. Although NETs were initially identified as an important component of innate immunity, they have subsequently been associated with numerous pathological conditions, highlighting their dual role as both protective and potentially harmful mediators.[9]

During NETosis, neutrophils release numerous enzymes from their granules into the extracellular space. These enzymes, including myeloperoxidase (MPO), play an important role in NET formation.[9-11]

It has been shown that inhibition of intragranular MPO activity or neutralization of intragranular MPO-modified ROS using luminol effectively blocks NET formation, indicating that ROS generation and MPO-mediated processes within intracellular granules are required for the initiation of NET formation.[12]

In addition, neutrophils undergoing NETosis may release cytoplasmic contents, including mitochondria, into the extracellular space. These components contribute to the inflammatory response and additional antimicrobial activity.[13] In vascular inflammatory diseases, persistent neutrophil activation and NET formation have been implicated in endothelial injury, oxidative stress, and the progression of atherosclerosis. This association is particularly relevant to MB, where evidence suggests that the arterial segment proximal to the bridge exhibits a higher incidence of atherosclerotic lesions, whereas the tunneled segment remains relatively protected from atherosclerosis despite the presence of endothelial functional alterations that may predispose to vasospasm and thrombosis.[2,7]

Myocardial bridging was hypothesized to create a spatially heterogeneous oxidative and inflammatory microenvironment driven by altered shear stress, which may favor MPO activation and potentially contribute to NET formation in the proximal coronary segment, thereby contributing to endothelial dysfunction, atherogenesis, and ischemic manifestations.

HIGHLIGHTS

- Myocardial bridging (MB) creates a spatially heterogeneous coronary microenvironment driven by altered shear stress and disturbed flow.
- The proximal segment exhibits enhanced oxidative stress, endothelial dysfunction, and inflammation mediated by myeloperoxidase-derived reactive oxygen species.
- Neutrophil extracellular trap formation is proposed as a previously underrecognized mechanism linking MB to proximal coronary atherogenesis and ischemic manifestations.
- Integrating hemodynamic and immune mechanisms may open new perspectives for targeted therapeutic strategies.

METHOD

Literature Search Strategy

This narrative review summarizes and integrates current evidence regarding the potential role of NETs and oxidative stress in the pathophysiology of MB. A structured literature search was performed using PubMed/MEDLINE and Scopus to identify relevant publications published from 1990 through May 2026. Search terms included combinations of MB, NETs, NETosis, MPO, oxidative stress, ROS, NO, endothelial dysfunction, shear stress, and atherosclerosis. Original research articles, experimental studies, clinical investigations, and relevant review articles published in English were considered. Emphasis was placed on studies investigating the mechanisms of endothelial dysfunction, redox signaling, MPO biology, NET formation, and coronary hemodynamics. Because direct evidence linking NETosis with MB is currently limited, mechanistic evidence from related cardiovascular and vascular inflammatory conditions was also incorporated to develop the proposed pathophysiological framework. This review follows a narrative rather than a systematic methodology; therefore, studies were selected based on their scientific relevance, methodological quality, and contribution to the mechanistic understanding of the topic, rather than on predefined systematic review criteria.

Pathophysiology of Myocardial Bridging

The pathophysiology of MB involves disruption of local vascular homeostasis through dysregulation of NO metabolism,

increased generation of ROS, and activation of oxidative-inflammatory pathways.[2] Wall shear stress (WSS), defined as the frictional force exerted by flowing blood tangentially on the luminal surface of the vessel wall, represents an important mechanical factor influencing the hemodynamic characteristics of the proximal and intramural segments of MB.

The pathophysiological mechanism involves dynamic compression of the tunneled segment during systole, causing localized disturbances in coronary blood flow and alterations in WSS within the proximal arterial segment, thereby creating a pro-oxidative and pro-inflammatory microenvironment that contributes to the development of atherosclerotic changes.[1,2,8,14]

Oxidative stress, resulting from the intracellular generation and accumulation of ROS, promotes lipid accumulation, foam cell formation, and extracellular matrix degradation. These oxidative modifications impair the anti-inflammatory and vasodilatory functions of the endothelium and serve as a signal for neutrophil activation.[15]

During chronic vascular inflammation associated with atherosclerosis, as well as during chronic occlusive coronary artery disease, MPO, an enzyme released from activated leukocytes, plays an important role. The MPO markedly reduces the bioavailability of NO, a key mediator of vascular homeostasis. It catalyzes the formation of hypochlorous acid (HOCl) from hydrogen peroxide (H_2O_2) and chloride ions, generating highly reactive oxidant species.[16]

The preferential development of atherosclerosis, chronic coronary syndrome, and chronic total occlusions within the proximal segment of MB[7,17,18] indicates the involvement of the aforementioned pathophysiological mechanisms and raises the question of a possible association between MPO, NETs, and MB, which has not yet been systematically addressed in the available literature. Although the role of MPO in endothelial dysfunction and atherosclerosis is well established, direct evidence linking MPO activity to MB is currently lacking. Consequently, the proposed involvement of MPO in MB should be interpreted as a mechanistic extrapolation based on established vascular biology rather than as an experimentally confirmed feature of MB.

In contrast to the hemodynamic profile of the proximal segment, the tunneled and distal arterial segments are characterized by elevated WSS. These conditions exert atheroprotective effects, including enhanced NO production, reduced platelet activation, and suppression of pro-inflammatory signaling pathways.[14,19] The established anatomical, hemodynamic, endothelial, inflammatory, and functional differences between the proximal and intramural coronary segments are summarized in Table 1, providing the pathophysiological framework for the subsequent discussion of oxidative stress, MPO activation, and NET formation.

Myeloperoxidase, Nitric Oxide Synthase, and Neutrophil Extracellular Traps: Role in Oxidative Stress in the Proximal Segment of the Myocardial Bridge

Disturbed blood flow in the proximal part of the myocardial bridge can damage and activate endothelial cells lining the arterial intima.[20,21] Neutrophils attracted by chemokines produced by activated endothelial cells may accumulate on the exposed intima, where they may subsequently undergo degranulation and NETosis, releasing NETs. These strands of extracellular DNA can bind the contents of neutrophil granules and other proteins, such as MPO or tissue factor. NETs may act as a "solid-phase reactor," generating oxidants such as HOCl and locally promoting coagulation.[22]

The generation of oxidants represents a positive feedback loop in vascular inflammation, in which the mutual activation of MPO, NADPH oxidase, and NOS is a key mechanism of NET formation.[23] NADPH oxidase provides the initial ROS, MPO catalytically converts H_2O_2 into potent oxidants, while NOS contributes by producing reactive nitrogen species.

This pro-oxidative integration of MPO, nicotinamid adenine dinulceotide phosphate (NADPH) oxidase, NOS, and NETs is known to reduce NO bioavailability, promote oxidative modification of lipoproteins, and consequently create a pro-inflammatory environment that promotes the progression of atherosclerotic lesions and plaque destabilization.[24,25]

Although oxidative stress and dysregulated lipid metabolism are well-established contributors to cornonary arthery disease (CAD), accumulating evidence increasingly highlights the central role of immune responses and inflammation in disease progression.[26]

At the molecular level, activation of NADPH oxidase represents a critical step in NETosis. This enzyme complex generates ROS, which are indispensable for NOX-dependent NET formation, as pharmacological or genetic inhibition of ROS production markedly suppresses this process.[27,28]

During neutrophil activation, ROS are generated predominantly by NADPH oxidase, while mitochondrial metabolism may provide an additional intracellular source through the electron transport chain.[29] The hydrogen peroxide (H_2O_2) produced downstream of NADPH oxidase serves as the principal substrate for MPO, which catalyzes the formation of HOCl, a highly reactive oxidant involved in antimicrobial defense as well as oxidative tissue injury.

Excessive pro-oxidative activity of MPO reduces the bioavailability of NO by promoting eNOS uncoupling, generating superoxides within the endothelium, and contributing to the progression of atherosclerosis.[3,30] Studies have reported decreased eNOS expression in severe atherosclerotic lesions of the coronary arteries. However, the significantly lower eNOS expression observed in the bridged, atheroprotective segment of the myocardial bridge remains a matter of controversy.[31]

Akong-Moore et al[32] conducted a study to determine the role of HOCl in NET formation. The results showed that reducing Cl^- or inhibiting MPO with the hydrazide 4-aminobenzoic acid decreased NETosis in humans. The addition of exogenous sodium hypochlorite (NaOCl) stimulated NET formation in a dose-dependent manner. Even when NET formation was pharmacologically inhibited by MPO blockade, supplementation with NaOCl alleviated the defect in NET formation.

Table 1. Evidence-Based Comparison of Proximal and Intramural Coronary Segments in Myocardial Bridging

Category	Proximal Segment (Upstream of Myocardial Bridge)	Intramural Segment (Within Myocardial Bridge)	Evidence Level
Anatomical characteristics	Predisposition to plaque formation due to disturbed flow proximal to the bridge	Intramyocardial artery compressed during systole	Established
Hemodynamic profile	↓ Endothelial shear stress (ESS); oscillatory WSS; ↑ tensile stress; increased wall motion	Normal/high ESS; high unidirectional WSS; low tensile stress; reduced wall motion	Established
Endothelial function	↓ Nitric oxide bioavailability; ↑ ACE expression; ↑ Endothelin-1; endothelial dysfunction	Relative preservation of endothelial structure despite functional endothelial alterations; eNOS/NO signaling remains controversial	Moderate evidence (conflicting data regarding eNOS/NO signaling)
Adhesion molecules	↑ ICAM-1; ↑ VCAM-1 expression	Reduced ICAM-1 and VCAM-1 expression	Moderate evidence
Inflammatory response	↑ TNF-α; ↑ IL-1; increased inflammatory signaling; ↑ neutrophil-to-lymphocyte ratio (NLR)	Reduced inflammatory activation	Moderate evidence (NLR: limited evidence, single study)
Oxidative stress	↑ Reactive oxygen species (ROS); oxidative stress	Relatively lower oxidative stress compared with the proximal segment	Moderate evidence
Proposed neutrophil-mediated mechanisms	Possible involvement of MPO, PAD4-mediated NETosis and NET formation contributing to oxidative stress and endothelial injury	No direct evidence of neutrophil activation or NETosis in the intramural segment	Hypothesis (no direct evidence in myocardial bridging)
Atherosclerotic phenotype	Frequent plaque formation; positive remodeling; low-attenuation plaques; spotty calcifications; occasional napkin-ring sign	Relative protection from atherosclerosis	Established (napkin-ring sign: limited evidence)
Protective mechanisms	None identified	High unidirectional shear stress; relative atheroprotection; role of eNOS/NO signaling remains controversial	Moderate evidence (presumed)
Functional significance	Reduced FFR/iFR; reduced CFR; inducible ischemia on stress imaging (stress echo, PET, CMR); regional wall motion abnormalities	Preserved coronary flow reserve in most patients	Established
Clinical manifestations	Angina; myocardial ischemia; arrhythmias; acute coronary syndrome	Usually asymptomatic unless severe systolic compression is present	Established

ACE, angiotensin-converting enzyme; CFR, coronary flow reserve; CMR, cardiac magnetic resonance; eNOS, endothelial nitric oxide synthase; ESS, endothelial shear stress; FFR, fractional flow reserve; ICAM-1, intercellular adhesion molecule-1; iFR, instantaneous wave-free ratio; IL-1, interleukin-1; MACE, major adverse cardiovascular events; MPO, myeloperoxidase; NETs, neutrophil extracellular traps; NLR, neutrophil-to-lymphocyte ratio; NO, nitric oxide; PAD4, peptidylarginine deiminase 4; PET, positron emission tomography; ROS, reactive oxygen species; TNF-α, tumor necrosis factor alpha; VCAM-1, vascular cell adhesion molecule-1; WSS, wall shear stress.

In another study, the role of MPO-mediated conversion of hydrogen peroxide (H_2O_2) and chloride ions (Cl^-) into HOCl was tested on peripheral human neutrophils, where experimental groups either contained or lacked extracellular Cl^-. NETosis was completely suppressed in the absence of extracellular Cl^-. The study revealed that depletion of Cl^- or inhibition of MPO significantly reduced NETosis in human samples.[33]

Microvascular disease and vasospasm, in combination with coronary atherosclerosis of the proximal MB segment, may represent a major contributor to myocardial ischemia in some patients, indicating that the spectrum of stable ischemic heart disease is overly simplified.

Hemodynamics of Myocardial Bridging in the Context of Proinflammatory Endothelial Activation

Myocardial bridging comprises 2 hemodynamic regions with different susceptibilities to atherogenesis. The proximal region of the MB is the segment of the coronary artery located immediately before the artery enters the tunneled portion, i.e., before the onset of muscular compression, while the intramural (tunneled) region represents the segment of the coronary artery that passes within the myocardium, compressed during systolic contraction and relaxed during diastole.

These hemodynamic alterations expose the proximal and intramural endothelial monolayers to distinct biomechanical

environments characterized by different shear stress magnitudes and flow patterns.[34] Intravascular ultrasound and autopsy studies consistently demonstrated a pronounced spatial heterogeneity of atherosclerosis across MB, with a preferential localization of atherosclerotic lesions in the proximal segment, while the intramural and distal segments remain relatively spared.[35]

Multiple cellular and molecular proatherogenic mechanisms have been described under conditions of low WSS, including the development of a proatherogenic endothelial cell phenotype[36] and an increase in circulating proatherogenic vasoactive and proinflammatory agents.[37,38,39]

As this proatherogenic phenotype progresses, endothelial cells within the proximal segment become flattened, polygonal, and structurally dysfunctional in response to persistent flow disturbances.[40]

The mechanism underlying the development of an atherogenic endothelial phenotype in the proximal segment of the myocardial bridge under conditions of low WSS is associated with increased expression of vascular cell adhesion molecule-1 (VCAM-1) and enhanced activity of NADPH oxidase and lipoxygenase, both of which contribute to ROS generation.[2] These molecular alterations promote the development of an atheroprone endothelial phenotype characterized by impaired antioxidative and anti-inflammatory defense mechanisms together with increased activation of proinflammatory and proatherogenic signaling pathways.

Within such a pro-oxidative environment, MPO, a well-established mediator of vascular inflammation and atherosclerosis, may further aggravate endothelial dysfunction through several complementary mechanisms, including inhibition of eNOS, depletion of NADPH and other cofactors required for NO synthesis, and direct oxidation of NO by MPO-derived reactive species, particularly HOCl. These mechanisms may reduce NO bioavailability and may contribute to the progression of atherosclerotic lesions in the proximal segment of the myocardial bridge. Beyond its effects on oxidative stress, MPO has also been shown to modulate immune responses by influencing macrophage differentiation, promoting the production of proinflammatory cytokines such as tumor necrosis factor alpha, and suppressing interferon-γ expression.[41]

It has been proposed that marked angulation of the vessel at the proximal junction, caused by systolic compression within the bridge, may promote endothelial injury and favor atherosclerotic plaque formation.[42]

It is hypothesized that the pro-oxidative microenvironment generated within the proximal segment of the myocardial bridge may facilitate neutrophil NADPH oxidase activation and oxidative burst–driven ROS production. Based on mechanisms established in NETosis, these processes may facilitate granule membrane and nuclear envelope destabilization, leading to the mixing of cytoplasmic and nuclear components. Consequently, cytoplasmic neutrophil elastase activation, histone cleavage, chromatin decondensation, and ultimately NETosis may occur.

This hypothetical mechanism is biologically plausible because activation of NADPH oxidase represents one of the major intracellular sources of ROS during neutrophil activation.

In activated neutrophils, NADPH oxidase generates superoxide anions and hydrogen peroxide, which are subsequently converted into highly reactive oxidant species, including hydroxyl radicals (•OH) and HOCl.[3,43]

A significant correlation has been documented between eNOS activity, NO bioavailability, and vascular WSS in humans in vivo.[31]

Immunohistochemical analyses have demonstrated segment–specific alterations in the expression of vasoactive molecules, including eNOS, endothelin-1 (ET-1), and angiotensin-converting enzyme (ACE), within MB. Reduced endothelial expression – particularly of eNOS – has been reported in the intramural segment compared with adjacent proximal and distal segments of the LAD.[31]

Based on the data, the reduced endothelial eNOS expression within the intramural segment appears inconsistent with its relative resistance to atherosclerosis. However, reduced eNOS expression does not necessarily indicate diminished NO bioavailability under physiological conditions. Endothelial function is determined not only by the amount of eNOS protein but also by its enzymatic activity, post-translational regulation, phosphorylation status, substrate and cofactor availability, and the local hemodynamic environment. In the intramural segment, exposure to elevated laminar shear stress during diastolic coronary perfusion may promote rapid activation of the existing eNOS pool and transient NO release despite lower overall eNOS expression. Consequently, endothelial function reflects the dynamic balance between eNOS expression and activation rather than protein abundance alone. This distinction may partly explain why the intramural segment remains relatively protected from atherosclerotic plaque formation despite reduced eNOS expression reported in histological studies.[31]

Hemodynamically, the proximal segment of MB is exposed to disturbed flow and reduced WSS, which is widely recognized as the principal driver of atherosclerotic plaque formation immediately proximal to the bridge, whereas plaque deposition within or distal to the tunneled segment remains infrequent.[42] Autopsy studies of patients with MB have shown structurally dysfunctional, flat, and polygonal endothelial cells in the segments proximal to the MB, whereas the endothelial cells lining the bridged segments remain intact.[42,44]

Previous investigations indicate that the mechanical stress imposed by systolic compression may enhance platelet activation and vasoreactivity, and in selected cases has been associated with coronary vasospasm or acute coronary syndromes.[45,46]

The role of vasoactive mediators, particularly eNOS and NO, within the intramural segment of the myocardial bridge, as well as the occurrence of paradoxical vasoconstriction in intact or damaged endothelium without the formation of

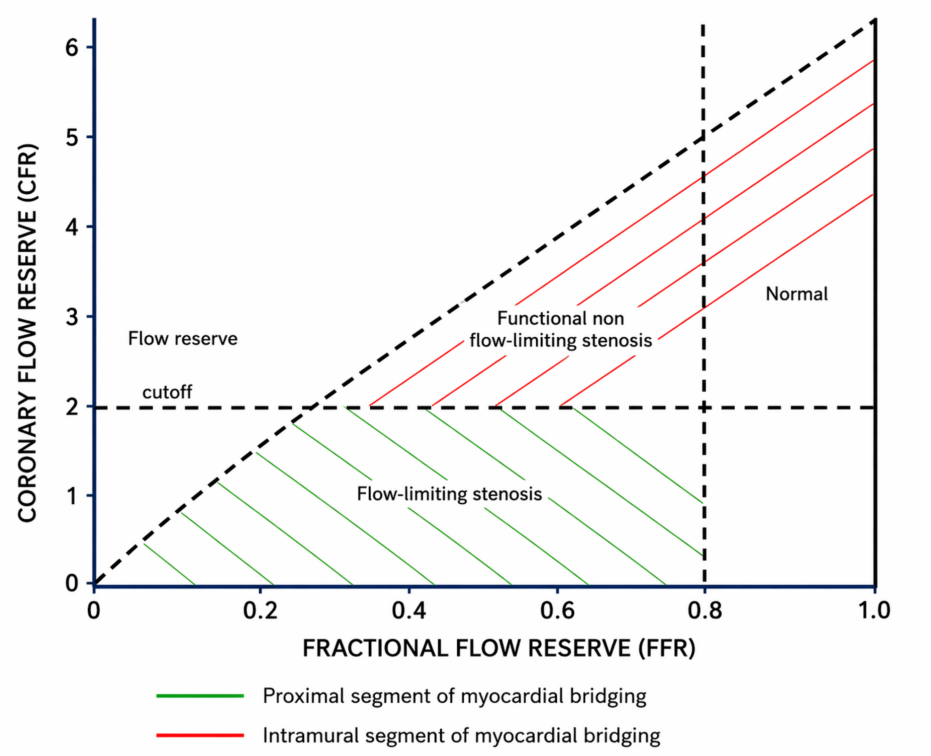


Figure 1. Functional severity of coronary stenosis in the proximal and intramural segments of myocardial bridging.

atherosclerotic lesions, remains controversial. Although this segment is typically considered relatively protected from atherosclerosis, the occurrence of paradoxical vasoconstriction in the absence of structural plaque formation suggests the presence of functional endothelial alterations.

One proposed explanation for this paradox of vasoconstriction and the positive response to acetylcholine in intact endothelium suggests that under conditions of markedly elevated shear stress, harmful increases in oxidative stress may override the protective effects that normally maintain the concentration of vasoactive agents (eNOS, NO) in the intramural segment of the MB.[42]

However, experimental data remain conflicting. In the study by Shiode et al,[47] acetylcholine induced a significant reduction in vessel diameter predominantly in the bridged segments of the left anterior descending artery (LAD), whereas N-methyl-L-arginine reduced the diameter of all LAD segments. These findings suggest that segment-specific differences in NO synthase activity are less likely to explain the variations in vasoreactivity.[42]

These divergent observations underscore the need for a more detailed mechanistic evaluation of endothelial function and redox signaling within the intramural segment of MB. Taken together, the available studies suggest that endothelial dysfunction in MB is more complex than changes in eNOS expression alone. Differences in study design, methods of endothelial assessment, and the dynamic influence of local hemodynamics likely contribute to the apparently conflicting observations reported in the literature.

The direct contribution of these inflammatory mechanisms to MB remains to be experimentally confirmed.

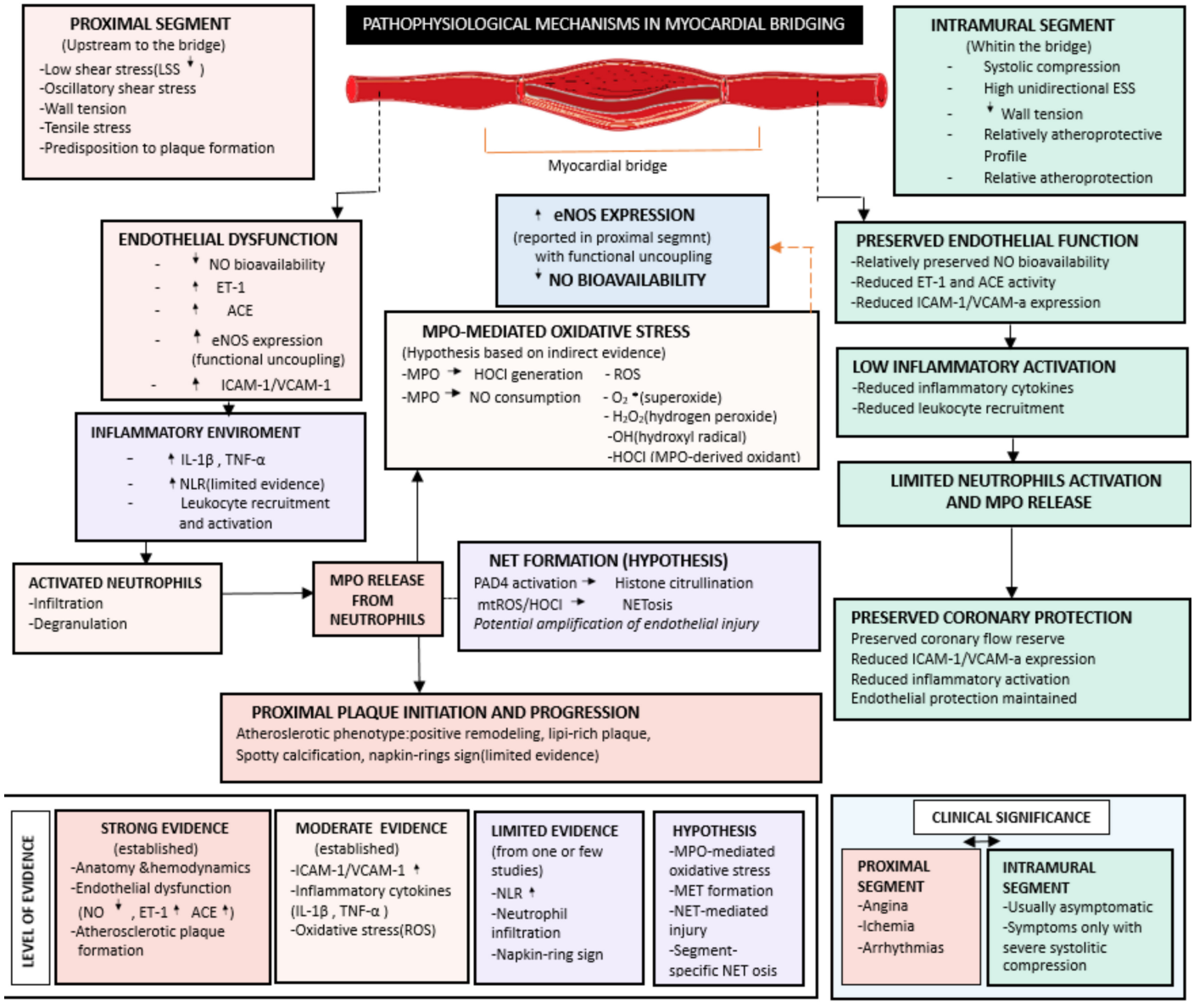
The functional consequences of these distinct hemodynamic environments are summarized in Figure 1, which illustrates the relationship between fractional flow reserve (FFR) and coronary flow reserve (CFR) in the proximal and intramural segments of MB and highlights their differing physiological significance. The proximal and intramural segments occupy different functional regions within the FFR–CFR relationship, emphasizing that anatomical compression alone does not necessarily predict physiological severity. The figure highlights the heterogeneous functional behavior of MB, which provides the hemodynamic basis for the proposed segment-specific oxidative and inflammatory mechanisms discussed below.

Netosis and the Inflammatory Response in the Proximal Segment of Myocardial Bridging

NETosis represents a key mechanism in the immune response mediated by neutrophils, characterized by 2 primary pathways: suicidal and vital NETosis. Suicidal NETosis is a specific form of cell death characterized by the release of NETs. This process begins when neutrophils encounter infectious agents or inflammatory stimuli such as bacteria, fungi, or chemical signals like PMA.[48] Activation of these neutrophils leads to the production of ROS via the NADPH oxidase complex, which is crucial for the progression of NETosis. ROS, MPO, and NE migrate from the granules into the nucleus, where they facilitate chromatin decondensation through histone processing.

The production of ROS plays a dual role—it aids in the elimination of pathogens within the NETs but, when excessive, ROS can be detrimental to surrounding tissues, causing inflammation and tissue damage.[49] Vital NETosis is a rapid, life-preserving process through which neutrophils release NETs to combat pathogens without undergoing cell death, distinguishing it from suicidal NETosis. This form of NET release can occur within minutes of exposure to infectious agents or inflammatory stimuli,[50] enabling a prompt immune response.

In the context of MB, the pro-oxidative microenvironment within the proximal segment, characterized by disturbed flow, reduced WSS, sustained endothelial activation, and increased ROS generation, may create favorable conditions for NETosis. Based on mechanisms established in vascular inflammation and atherosclerosis, prolonged oxidative stress and persistent neutrophil activation may create conditions compatible with NOX-dependent NET formation. However, direct evidence supporting this mechanism in MB is currently lacking and requires experimental validation. The mechanism of inflammation and atherogenesis in the proximal segment of the myocardial bridge under the influence of low shear stress involves the expression of ICAM-1, VCAM-1, and E-selectin, as well as increased production of ROS in the endothelium. Adhesion molecules facilitate the attachment and transmigration of monocytes and neutrophils in the proximal segment of the myocardial bridge, creating conditions for local inflammatory activation. Clinical



Abbreviations:ACE-angiotension-converting enzyme; eNOS-endothelial nitric oxide synthase; ET-1-endothelin-1; ICAM-1-intercellular adhesion molecule-1; VCAM-1-vascular cell adhesion molecule-1: MPO-myeloperoxidase;NETs-neutrophil extracellulartraps;NO-nitric oxide;NLR-neutrophil-to-lymphocyte ratio; ROS-reactive oxygen species; ESS-endothelial shear stress; PAD4-peptidylarginine deiminase 4;mtROS-mitochondrial ROS; HOCl- hypochlorous acid.

Figure 2. Potential pathophysiological mechanisms in myocardial bridging.

evidence of endothelial dysfunction, altered vasoreactivity, and elevated inflammatory markers supports the presence of localized vascular inflammation in patients with MB.[21]

When MPO is released into circulation, it adsorbs onto negatively charged surfaces such as the endothelium or lipoproteins. The presence of chlorinated apolipoprotein B-100 extracted from human lesion material indicates a direct involvement of the MPO–H_2O_2–Cl^- system with low-density lipoprotein (LDL) as one of the main targets of oxidative modification, emphasizing its potential role in the development of atherosclerosis.[4]

Figure 2 summarizes the proposed mechanistic framework linking altered coronary hemodynamics, oxidative stress, endothelial dysfunction, MPO activation, and NET formation in MB. It integrates experimentally established mechanisms with hypothesized MB-specific pathways and is intended to guide future mechanistic investigations rather than represent an experimentally validated pathway. The strength of evidence supporting the proposed mechanisms varies considerably. Evidence linking altered coronary hemodynamics and proximal atherosclerosis to MB is relatively strong, whereas evidence for MPO- and NET-mediated mechanisms in MB is largely indirect and extrapolated from vascular inflammation and atherosclerosis. Direct evidence of NET formation or MPO-driven NETosis specifically in MB is currently lacking.

CONCLUSION

The interplay between MPO, NETs, eNOS, and NO, as key mediators of endothelial dysfunction, inflammation, and atherosclerosis, may represent a mechanistic link between the pathogenesis of MB and its clinical manifestations. A better understanding of these pathways would contribute to the development of more comprehensive diagnostic and therapeutic strategies for MB, whose underlying biological mechanisms remain incompletely understood.

In contrast, the intramural segment of the bridged coronary artery is exposed to intermittently elevated WSS, creating a specific biomechanical environment that contributes to the paradoxical vasomotor response to acetylcholine. Such a response differs from its conventional prognostic significance in atherosclerosis and suggests the existence of distinct local regulatory mechanisms.

Despite advances in understanding the atherosclerotic and non-atherosclerotic mechanisms contributing to the clinical manifestations of MB, several important questions remain unresolved. These include the diverse roles of ROS, the conditions favoring activation of distinct NETosis pathways, immune responses mediated by infiltrating neutrophils, as well as the complex interactions among MPO, eNOS, and NO. The proposed integration of these pathways highlights previously underrecognized links between coronary biomechanics, innate immunity, and vascular pathology.

Although direct evidence supporting a significant role of MPO and NETs in the pathophysiology of MB remains limited, their well-documented involvement in endothelial dysfunction, oxidative stress, chronic vascular inflammation, and atherosclerotic plaque development, particularly within the proximal arterial segment, provides a biological rationale for further investigation of these mechanisms in the context of MB.

The proposed framework may also have clinical relevance, although these implications remain to be confirmed. A combination of anatomical and functional assessment, including measures of coronary flow and vasoreactivity, may help identify patients in whom MB is clinically significant. Future studies should determine whether markers of neutrophil activation, MPO activity, or NET formation could add useful information for risk stratification.

Patients with MB are clinically heterogeneous, ranging from largely asymptomatic individuals to those with endothelial dysfunction, vasospasm, proximal atherosclerosis, or recurrent ischemic symptoms. Better characterization of the underlying biological phenotype may therefore improve prognostic assessment and help guide management.

At present, treatment remains focused on reducing ischemia and hemodynamic stress, with invasive approaches reserved for selected symptomatic patients. Whether inflammatory or oxidative pathways could become therapeutic targets in specific subgroups remains an open question and will require direct clinical validation.

Overall, the available evidence strongly supports the contribution of altered coronary hemodynamics to endothelial dysfunction in MB, whereas the proposed involvement of MPO- and NET-mediated mechanisms remains hypothesis-generating and should stimulate future mechanistic investigations.

Ethics Committee Approval: This study was approved by the Etichs Committee of the Public Institution Health Center of Sarajevo Canton (Approval No: 01-06-33-2-1844-2/23, Date: 08 June 2023).

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