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Review

FMD for the Masses? A Realistic Look at Endothelial Function Assessment and Its Limits

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Abstract

Background: Research on the vascular endothelium matters because its dysfunction—driven by reduced nitric oxide (NO) synthesis or bioavailability—plays a pivotal role in the early pathogenesis of atherosclerosis. A standard method for assessing endothelial function is to measure flow-mediated dilation (FMD) of the brachial artery, induced by prior occlusion of the forearm arteries or the proximal portion of the arm. **Methods:** This review synthesizes current evidence on the physiological basis of flow-mediated dilation (FMD), emphasizing contemporary consensus methodology and the contextual boundaries of result interpretation. **Results:** In research frameworks, FMD applications primarily center on evaluating cohort-level cardiovascular risk associations, monitoring endothelial responses to vasoprotective pharmacotherapies, and exploring underlying vasodilatory mechanisms in conditions such as neurocardiogenic syncope, rather than serving as a routine bedside diagnostic modality. **Conclusions:** FMD is a highly effective research instrument for population-level epidemiology, clinical trials, and small mechanistic studies. However, it is not suitable for routine individual assessment in everyday clinics. Biological variability, mandatory pre-test resting periods, and limited access to specialized hardware such as stereotactic probe Fs and dedicated armrests hinder practical adoption at the bedside. The method is limited by its diagnostic value and a lack of evidence for FMD-guided therapy, which keeps it anchored in specialized laboratories.

Keywords: flow-mediated dilation; endothelial dysfunction; guidelines; nitric oxide; vascular pathology; methodological standardization

1. Introduction

Understanding vascular endothelial physiology is essential, largely because endothelial dysfunction—driven by impaired nitric oxide (NO) synthesis or diminished bioavailability—acts as a primary trigger in early atherogenesis [1,2]. This mechanism underpins the concept that assessing endothelial function can offer valuable insights into the trajectory of atherosclerotic disease (Figure 1). Because endothelial vasomotor activity actively regulates local blood flow, it shifts noticeably with an individual's burden of cardiovascular risk factors [1,3]. Consequently, contemporary vascular diagnostics combine morphological, functional, and biochemical metrics to capture a more complete picture of arterial wall structure and mechanical performance [3].

The modern diagnostic panel for evaluating the functional and structural status of the vascular endothelium includes both invasive and noninvasive techniques. These methods are classified by two criteria: the nature of the parameters assessed (functional vs. structural) and the measurement methodology (physical vs. biochemical) (Table 1).

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Table 1. Research methods for assessing endothelial function.

Invasive Functional Tests	
Quantitative coronary angiography with an acetylcholine (ACh) provocation test	[4–6]
Intravascular ultrasound (IVUS) with an ACh provocation test	[5–7]
Forearm occlusion plethysmography (forearm blood flow—FBF) with intra-arterial administration of endothelial agonists	[5,7]
Doppler measurement of intravascular blood flow velocity in the coronary arteries	[1,5]
Noninvasive functional tests:	
Ultrasound measurement of flow-mediated dilation (FMD)	[6–8]
Peripheral arterial tonometry (PAT/EndoPAT)	[5–7]
Laser Doppler flowmetry (LDF) with iontophoresis (ACh, sodium nitroprusside)	[5]
Laser speckle contrast imaging (LSCI)	[9]
Thermally induced vasodilation (thermal hyperemia)	[10,11]
Noninvasive structural and morphological methods:	
Capillaroscopy of the nailfoldes	[12–16]
Intima–media thickness (IMT) measurement of arterial vessels	[17–19]
Biochemical (plasma and urine biomarkers):	
asymmetric dimethylarginine (ADMA) von Willebrand factor (vWF) endothelin-1 (ET-1) concentrations of nitric oxide and its stable metabolites soluble type I cell adhesion molecules (sICAM-1) soluble vascular cell adhesion molecule-1 (sVCAM-1) E-selectin, P-selectin circulating endothelial progenitor cells (EPCs) circulating endothelial microparticles (EMP) microalbuminuria in a 24 h urine collection	[5,20–22]

The established noninvasive approach to evaluating endothelium-dependent regulation of vascular tone is to measure flow-mediated dilation (FMD) of the brachial artery, induced by prior, controlled ischemia resulting from occlusion of the arteries of the forearm or the proximal arm. The physiological basis of the arterial response to ischemia was first described by Schretzenmayr in 1933, who demonstrated that conduit arteries widen in response to heightened blood flow [23]. However, the use of modern FMD imaging as a sensitive indicator of endothelial dysfunction was achieved decades later by Celermajer and colleagues [24].

Mechanistically, the rapid increase in blood flow after cuff release during an FMD test increases shear stress on the endothelial cell layer. This, in turn, directly reduces vascular wall tension [23,24]. Short-term exposure to elevated shear stress has been shown to open calcium-activated potassium channels in endothelial cells [23].

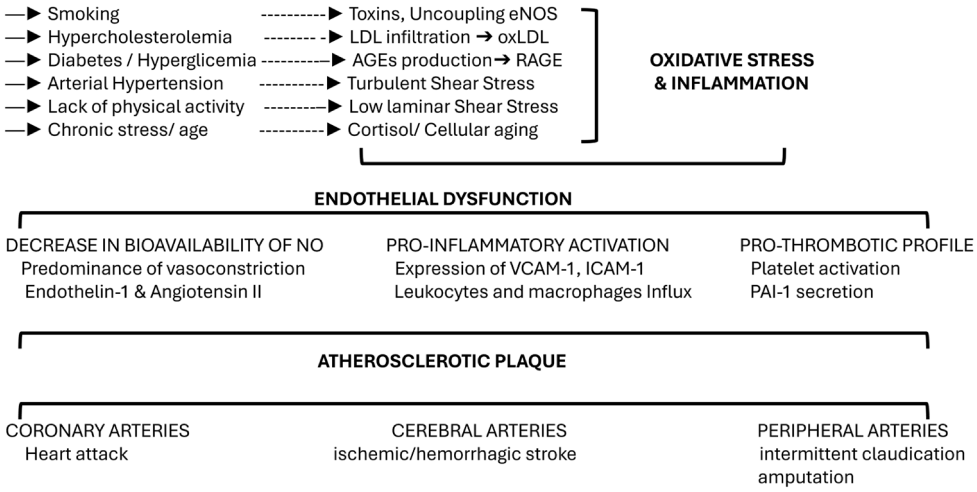


Figure 1. Endothelial Dysfunction: Correlations, Causes, and Consequences [25,26].

This process hyperpolarizes the cell membrane and stimulates calcium influx into the intracellular matrix, thereby activating the endothelial isoform of nitric oxide synthase

(eNOS) [3,27]. Prolonged exposure to elevated shear forces (lasting from several minutes to several hours) predominantly activates the NOS3 gene (encoding eNOS), resulting in a sustained increase in NO (nitric oxide) bioavailability [3,24]. Long-term shear stress also stimulates NO secretion via direct eNOS phosphorylation, which occurs independently of increases in intracellular calcium ion concentrations [24]. Ionic parameters of the cellular environment modulate this response: reductions in intracellular calcium and sodium concentrations have been correlated with a weaker response in the FMD test, whereas magnesium supplementation has been documented to optimize vascular reactivity [3,24].

The final percentage value of the FMD index reflects many interrelated biological processes (Figure 2). The test result depends not only on the rate of NO synthesis and extent of inactivation, but also on vascular smooth muscle cell sensitivity to NO and the degree of reduced perfusion in a structurally or functionally impaired microcirculation [1].

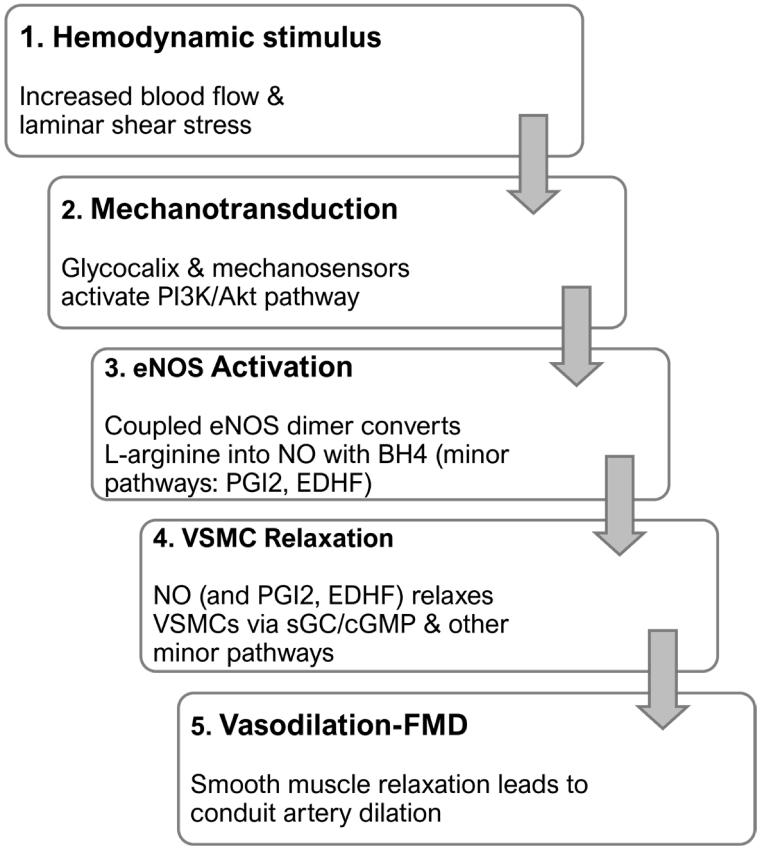


Figure 2. A simplified physiological cascade of flow-mediated dilation (FMD) in a conduit artery. Increased blood flow generates laminar shear stress on the endothelial surface (1), triggering mechanotransduction via the glycocalyx and endothelial mechanosensors to activate the phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) signaling pathway (2). Subsequent activation of the coupled endothelial nitric oxide synthase (eNOS) dimer converts L-arginine into nitric oxide (NO) in the presence of the cofactor tetrahydrobiopterin (BH4), alongside minor vasodilatory pathways involving prostacyclin (PGI2) and endothelium-derived hyperpolarizing factor (EDHF) (3). Diffusing NO stimulates soluble guanylate cyclase (sGC) within adjacent vascular smooth muscle cells (VSMCs), elevating intracellular cyclic guanosine monophosphate (cGMP) levels (4) and inducing smooth muscle relaxation that results in measurable conduit artery dilation (5).

To obtain a comprehensive profile of the progression of vascular changes, dynamic FMD assessment is combined with measurements of arterial wall physical properties and macroscopic structure. The following diagnostic techniques have been shown to provide important clinical information in this context [3]:

- Measurement of intima–media thickness (IMT) enables quantitative assessment of early morphological changes associated with atherosclerotic remodeling and intimal hyperplasia.
- Arterial stiffness is assessed using pulse wave velocity (PWV) to quantify the mechanical compliance and elasticity of the large arteries.
- An analysis of vascular remodeling indices is conducted to characterize the direction and extent of established structural vascular remodeling.

Physical diagnostics and imaging techniques are supplemented by laboratory measurement of serum or plasma concentrations of soluble markers of endothelial dysfunction [3]. Integrating methods that assess biochemical indicators of vascular activation, morphological parameters (IMT), and arterial wall mechanical function (PWV, FMD) provides a unique opportunity to obtain a comprehensive description of a patient's vascular system. This approach has broad applications in both scientific research and individualized clinical risk stratification [1,3].

While consensus documents [2,3,28–33] have standardized FMD, and multicenter analyses have begun mapping reference intervals, a central practical question remains: is this method ready for wide clinical use? Existing guidelines focus heavily on acquisition mechanics in traditional cardiovascular cohorts, often omitting newer drug therapies and emerging interdisciplinary fields. This review addresses that question. By pairing modern technical standards with a critical look at FMD under newer treatments—such as PCSK9 inhibitors and soluble guanylate cyclase stimulators—alongside its exploratory use in vascular surgery, perinatology, and neurology, we evaluate whether the test can truly scale. Ultimately, this synthesis explains why strict technical demands and the lack of universal cutoffs still block clinical adoption, keeping FMD locked in specialized research.

2. Literature Search Strategy

To ensure a rigorous, transparent, and reproducible evidence-gathering process, a systematic literature search was conducted across three major electronic databases: PubMed/MEDLINE, Scopus, and the Web of Science Core Collection. The search strategy was designed to identify peer-reviewed original research, clinical guidelines, and expert consensus documents that evaluate the methodological or clinical applications of flow-mediated dilation (FMD).

The search architecture relied on a combination of controlled vocabulary and targeted free-text keywords in titles and abstracts. In PubMed, the exact search strategy was implemented as follows: Search for "flow-mediated dilation" or "FMD." Search for "endothelium" in the MeSH Terms or Title/Abstract field. Use the search term AND ("Guideline"[Publication Type] OR "Practice Guideline"[Publication Type] OR "guideline*"[Title/Abstract] OR "consensus"[Title/Abstract] OR "recommendations"[Title/Abstract]). This strategy was adapted with corresponding syntax modifications for use in Scopus and Web of Science.

The electronic search yielded 411 records, distributed across the databases as follows: 132 articles from PubMed, 179 from Scopus, and 100 from Web of Science. During the database phase, strict filters were applied to limit retrieval to human studies published in English. After collecting the 411 records, we performed automated and manual deduplication. After removing duplicates, we identified 62 unique, high-quality papers, guidelines, and pivotal trials. We reviewed these in full text and synthesized them qualitatively in this manuscript.

3. Flow-Mediated Arterial Dilation (FMD)

Ultrasound measurement of flow-mediated dilation is one of the most widely recognized noninvasive methods for assessing endothelial function in blood vessels of the

peripheral circulation [2,33]. The literature indicates that this parameter has significant prognostic value for assessing cardiovascular event risk.

A pivotal step toward incorporating FMD into routine clinical practice was the dissemination of international guidelines and recommendations grounded in scientific evidence [34]. This document ultimately resolved long-standing methodological disputes by establishing rigorous criteria to standardize the procedure.

The primary objective was to establish a clear definition of the optimal location for the blood pressure cuff. The use of a sphygmomanometer on the distal forearm has become the prevailing standard. It has been demonstrated that, in contrast to the proximal position (on the upper arm)—which generates a stronger but less specific response—the distal approach minimizes the systemic neurohormonal response and the release of systemic tissue mediators. Consequently, the evaluation of this method focuses on isolating local endothelial nitric oxide (NO) synthesis. NO is generated in response to an increase in shear stress caused by the release of a flow obstruction.

The second key aspect highlighted in the consensus was the technical reduction in measurement error. Preliminary experimental studies have demonstrated that the conventional, manual assessment of vessel diameter using ultrasound cursors is associated with substantial operator variability and reduced reproducibility. Consequently, contemporary guidelines unequivocally advocate the exclusive use of automated software specifically designed for continuous, real-time monitoring of the vessel wall edge (edge detection systems). Assessing reproducibility means distinguishing structural baseline measurements from dynamic physiological responses. The automated tracking of the baseline brachial artery diameter yields high precision, with a coefficient of variation (CV) between 2.0% and 3.5%. On the other hand, the calculated FMD index exhibits significantly greater variability. Replicating the 3–4% error margin is unrealistic. Empirical data show that the actual FMD CV ranges from 11.6% to 16.1% in healthy volunteers and widens to 15.8–17.5% in cardiovascular patient cohorts [6,35,36].

3.1. Research Methodology and Standardization of Procedures

To ensure optimal reproducibility and reliability of the results, it is essential to adhere strictly to a detailed patient preparation protocol:

- Fasting requirement: Fasting for at least 12 h [37];
- Medication: Individualize vasoactive drug withdrawal based on specific compound half-lives. If withdrawal is clinically unsafe, schedule the FMD test strictly during the medication's trough plasma concentration window.
- Nicotine abstinence: Refrain from smoking tobacco products for at least 6 h before the start of the procedure.
- Environmental conditions: Provide a quiet room with a stable room temperature (20–22 °C).
- Hemodynamic stabilization: Maintain a supine position for at least 10 min immediately before the start of the examination.
- When evaluating upper extremity vessels, the brachial artery should be visualized in a longitudinal projection in two-dimensional (2-D) mode. Detection is performed using a linear probe with a frequency of 7–12 MHz [29]. Ultrasound imaging requires synchronous, continuous electrocardiographic (ECG) monitoring.

The FMD study protocol consists of the following stages:

$$FMD\% = \frac{D_{peak} - D_{baseline}}{D_{baseline}} \times 100\%$$

$D_{baseline}$—artery diameter before ischemia (baseline).

D_{peak}—artery diameter after ischemia (maximum dilation).

- Reactive hyperemia: The sphygmomanometer cuff is placed on the distal forearm. The cuff is inflated to 50 mmHg above the patient's systolic blood pressure and maintained to control ischemia for 5 min.

Note about the method: When the cuff is placed on the arm, it elicits a larger response in the blood vessels (higher flow and more pronounced dilation) than when it is placed on the forearm.

- Post-decompression measurement [6,29–31]: Perform continuous, longitudinal B-mode ultrasound imaging of the brachial artery for 180 s immediately following rapid cuff deflation. Rigid, fixed-time measurements (e.g., at 60 s) are prohibited due to chronological variability in time-to-peak dilation. Capture vessel diameter and blood flow velocity profiles simultaneously. Continuous concurrent tracking is mandatory to compute the dynamic shear-rate stimulus driving endothelial nitric oxide release.

Synchronize static frame extraction to end-diastole using the R-wave peak to minimize pressure-induced vessel distension. T-wave gating corresponds to early systole and is not interchangeable with R-wave parameters. Formal ECG gating is optional only when using validated, high-frequency continuous edge detection and automated wall-tracking software.

3.2. FMD Cutoffs

Recent epidemiological studies and prospective cohort analyses have provided strong evidence that a reduced FMD index is an independent prognostic factor for major vascular events. A comprehensive meta-analysis combining data from multicenter, long-term studies provides a better understanding of this risk profile [38]. Each 1 percentage-point increase in baseline brachial artery FMD is associated with a 13% lower risk of myocardial infarction, stroke, or cardiovascular death (RR, 0.87; 95% CI, 0.83–0.91) [38]. This predictive value has naturally drawn attention to treating FMD as a dynamic therapeutic target. Tracking changes in vascular reactivity over time provides more clinical information than relying on single, static measurements. When patients show a significant long-term increase in FMD following pharmacotherapy or lifestyle modifications, their long-term prognosis typically improves significantly. This phenomenon is often referred to as the reversal of endothelial dysfunction. Patients exhibiting biological resistance, in whom the FMD index remains unchanged despite optimal medical therapy in accordance with guidelines, typically have higher rates of rehospitalization and accelerated atherosclerotic progression. In this specific high-risk subgroup, targeting the nitric oxide pathway using a soluble guanylate cyclase stimulator, such as vericiguat, is a promising therapeutic approach. Preliminary evidence shows that vericiguat significantly improves endothelial dysfunction. Moreover, initial data indicate that it can enhance the response to cardiac resynchronization therapy (CRT) in individuals previously classified as non-responders due to endothelial dysfunction. This effect is the result of reduced levels of markers of inflammation and oxidative stress and lower endothelin-1 (ET-1) levels. By modulating these pathways, pharmacotherapy can restore functional myocardial resynchronization and stabilize the FMD index, reducing the risk of exacerbation of the disease [39].

Insights from landmark, population-based studies—drawing on both the long-term Framingham Heart Study and extensive Asian cohorts—have been instrumental in shaping our current understanding of FMD reference ranges, even though a universal consensus on clinical cutoffs remains elusive [40].

A major challenge in evaluating endothelial function via Flow-Mediated Dilation (FMD) is the substantial heterogeneity in established diagnostic thresholds. Historically, prospective data from classic epidemiological cohorts defined optimal cutoffs at 7.2% for

men and 6.2% for women [41]. However, contemporary reference guidelines tailored to European populations suggest a lower average threshold of 6.5% to denote preserved vascular health [40]. Within this European cohort, clear sexual dimorphism is evident, with reference values set at 5.7% for women and 6.7% for men [40]. These disparate cutoffs stem directly from differences in study populations, baseline geometric parameters, and institutional measurement protocols. For instance, the higher mean FMD responses often seen in women during screening (such as the Framingham Heart Study averages of 3.3% in women versus 2.4% in men) are largely driven by smaller baseline brachial artery diameters, which experience higher shear stress at equivalent flow velocities. Consequently, applying a rigid, single universal marker—such as the historically proposed ≥7.1% [41] —frequently fails to account for these physiological and methodological nuances.

To address these discrepancies, recent consensus documents propose a transitional zone between 4.0% and 7.0%, signaling an intermediate risk category that requires a comprehensive assessment of the patient's overall cardiovascular profile. FMD values consistently below 4.0% indicate overt endothelial dysfunction and early-stage atherogenesis. For clinical clarity, these reactive thresholds must be paired with endothelium-independent assessments. A post-nitroglycerin (0.4 mg sublingual) vasodilation of ≥15.6% is widely used to confirm preserved smooth muscle cell reactivity and effectively rule out structural arterial wall stiffening [41]. A detailed breakdown of these cutoffs, including their specific cohorts of origin and methodological limitations, is provided in Table 2.

Table 2. Flow-mediated dilation index cutoffs.

Study/Source	Study Cohort and Sample Size	Thresholds and Gender-Specific Metrics
Multicenter Japanese Cohort [41]	7277 Japanese adults (5817 men, 1460 women) from the multicenter FMD-J registry, healthy subjects without risk factors vs. patients with risk factors or CAD	normal FMD ≥ 7.1% (≥7.2% in men; ≥6.2% in women) normal NMD ≥15.6% (≥15.1% in men; ≥16.5% in women)
Japanese Risk Stratification [42]	5314 Japanese patients from general and clinical cohorts with varying CV risk profiles	FMD - 7.32 ± 3.46% without CV risk
Vascular Failure Criteria [43]	Expert consensus statement by the Japan Society for Vascular Failure (no original cohort)	normal ≥ 7.0% gray zone 4.0% to 6.9% abnormal < 4.0%
European Reference Data [40]	1579 healthy European adults (aged 18 to 76 years; 6 centers, 44 studies) alongside a CAD comparator group	optimal > 6.5% impaired 3.1–6.5% pathological < 3.1%
Age- and Sex-Specific Percentiles [44]	5362 multi-ethnic participants, including 1403 healthy adults (821 men, 582 women) free of CV risk factors	50th percentile: women/men at age 20: 8.7% and 7.8% at age 40: 6.8% and 6.7% at age 60: 5.0% and 5.6%
Methodological Consensus [6,31]	International expert guidelines (no original cohort)	No universal numeric cutoff; guidelines advise reporting baseline arterial diameter and shear rate to account for anatomical sex differences

3.3. Clinical Context and Applications of FMD Testing

3.3.1. Risk Stratification for Cardiovascular Events

The prognostic utility of FMD has been explored across high-risk cohorts. Its predictive strength depends on the clinical context and statistical adjustment. For instance, in a study tracking 444 high-risk patients over a median of two years, dropping below an FMD threshold of 2% correlated with a significantly higher incidence of cardiovascular events compared to individuals maintaining values at or above this mark [11]. FMD correlates strongly with invasive measurements of coronary endothelial function and discriminates coronary endothelial dysfunction, with an area under the ROC curve of 0.78. This confirms its usefulness as a noninvasive indicator of coronary endothelial health [45]. However, FMD did not emerge as an independent predictor of mortality once structural vascular markers—such as carotid intima–media thickness—were accounted for in multivariable models. Similarly, a brachial artery FMD lower than 4.5% is an independent predictor of

early cardiovascular complications following vascular interventions. This underscores its utility in identifying high-risk patients before surgery [46]. Askin et al. proved that patients with CSF are characterized by significantly higher IMT, impaired FMD, and significantly reduced blood flow velocity parameters in the carotid arteries. They suggested that a reduced FMD index indicates the need for preventive interventions [19].

3.3.2. Monitoring the Effect of Pharmacological Therapies on the Vascular System

Because endothelial status is dynamic, FMD is often used to track the effectiveness of vasoprotective drugs in alleviating vascular dysfunction. A meta-analysis of 35 studies ($n = 2178$) showed that statin therapy increases FMD by an average of 1.7% ($p < 0.001$), although significant heterogeneity persists, primarily due to differences in patients' baseline clinical status. Dose–response modeling within this dataset showed the greatest improvement in FMD at 20 mg/day of atorvastatin and 80 mg/day of simvastatin [47]. Newer lipid-lowering drugs, like PCSK9 inhibitors. They do not have a uniform response across the entire drug class. It is clear that alirocumab has a minimal effect on vascular reactivity. On the other hand, evolocumab results in a statistically significant improvement in brachial artery dilation. The pooled effect for the entire class of PCSK9 inhibitors was not statistically significant [48]. In the context of antihypertensive monotherapy, angiotensin-converting enzyme inhibitors (ACEIs) are the most effective in optimizing endothelial function. They outperform angiotensin II receptor antagonists. In combination therapy, the greatest enhancement in vascular dilation parameters is achieved with the concurrent administration of angiotensin II receptor blockers (ARBs) and calcium channel blockers [8].

3.3.3. Pathophysiological Aspects of Neurocardiogenic Syncope

Although FMD is not a diagnostic tool for neurocardiogenic syncope, assessing vascular reactivity sheds light on its underlying physiology. Patients with this condition often exhibit significantly greater flow-mediated dilation of the brachial artery than control subjects. This hyperactivity highlights the marked peripheral vasodilation that characterizes this disorder. Increased activity of the parasympathetic nervous system (PNS), particularly excessive vagus nerve activation, promotes acetylcholinergic transmission, directly inducing pronounced nitric oxide (NO)-dependent vasodilation [49].

4. Discussion

4.1. Clinical Correlations of Vascular Parameters

FMD indices, IMT, and PWV are recognized noninvasive parameters that reflect atherosclerosis severity and are used to assess cardiovascular risk [50]. However, the correlations among these parameters are not fully understood and depend on study participants' age and the clinical manifestation of atherosclerosis.

In a study of 34 men with overt atherosclerosis and 33 men without clinical symptoms (mean age: 61 ± 2 years), researchers found a statistically significant negative correlation between FMD and IMT [51]. Conversely, an analysis of 1578 relatively healthy men (mean age: 49 ± 10 years) found no association between brachial artery FMD and IMT [52].

Irace et al. reached similar conclusions when examining results from 55 men and 22 women [53]. Their analysis of classical risk factors—such as hypertension, diabetes, hyperlipidemia, and obesity—on FMD and IMT values indicated that FMD is sensitive to short-term changes and can serve as an early indicator of the efficacy of therapeutic interventions. In contrast, IMT is more useful for long-term monitoring of atherosclerotic structural progression.

In patients with mild hypertension, impaired endothelial function, as assessed by the FMD test, correlates with an increase in left ventricular mass index (LVMI), even within

the normal range [54]. This finding suggests that vascular dysfunction, characterized by impaired dilation, precedes structural organ complications and is closely associated with the pathophysiology of adverse myocardial remodeling.

Research indicates that in patients with heart failure [2], reduced FMD is a significant prognostic indicator for major adverse cardiovascular events (MACE). The predictive value of FMD is independent of the concentrations of N-terminal pro-B-type natriuretic peptide (NT-proBNP) or brain natriuretic peptide (BNP), as well as other clinical risk factors associated with unfavorable prognoses [55]. The substantial prognostic potential of FMD is attributed to its ability to reflect the cumulative impact of numerous interconnected pathophysiological processes [56–59]. These processes include the following:

- Decreased peripheral blood flow.
- Activation of proinflammatory cytokines.
- Enhanced activity of the renin–angiotensin–aldosterone system (RAAS).
- Increased oxidative stress.
- Elevated secretion of endothelin-1 (ET-1).

In a population with low baseline cardiovascular risk, endothelial function assessed by FMD shows a significant correlation with major risk factors and with the estimated 10-year probability of developing heart disease. Patients diagnosed with endothelial dysfunction using this method require rigorous monitoring and modification of the factors responsible for vascular damage, particularly hyperlipidemia, disorders of carbohydrate metabolism, hypertension, and smoking [60].

Kobayashi et al. [50] demonstrated that a decrease in brachial artery FMD is associated with increased PWV between the heart and the neck and with increased intima–media thickness. A positive correlation was also observed between PWV and IMT.

From a pathophysiological perspective, it is essential to validate the close relationship between the dynamic decline in FMD, the structural increase in IMT, and the rise in arterial stiffness as measured by PWV. Integrating these three assessments—functional (FMD), morphological (IMT), and hemodynamic (PWV)—provides a comprehensive characterization of the patient's vascular phenotype, thereby enhancing diagnostic precision and patient management strategies [61,62].

4.2. Interdisciplinary Research Frameworks and Limitations

While the primary application of FMD remains in academic cardiology, recent exploratory research has sought to map peripheral endothelial health across adjacent disciplines, including vascular surgery, perinatology, sports medicine, and neurology. However, these emerging data represent cohort-specific, observational associations. These findings do not constitute validated diagnostic pathways for routine bedside medicine.

In vascular surgery, observational cohorts have highlighted a potential prognostic role for FMD in specific high-risk scenarios. For instance, data suggest that an FMD below 4.5% may correlate with a higher incidence of early perioperative cardiovascular events after invasive peripheral vascular reconstructions [63]. Similarly, there is a clear link between brachial FMD and the annual expansion rate of the aneurysm sac in cases of abdominal aortic aneurysm. Mechanistically, this has led researchers to hypothesize that systemic endothelial dysfunction may reflect broader extracellular matrix degradation within the aortic wall; however, FMD is not used clinically to monitor aneurysm dynamics [64].

Perinatological research has similarly used FMD as a surrogate marker to investigate the early pathophysiology of gestational complications. Longitudinal studies indicate that blunted vascular reactivity can be detected in the second trimester among women who later develop preeclampsia, well before overt clinical signs such as hypertension or proteinuria appear [65,66]. Notably, this endothelial impairment may persist for up to three

years postpartum in some cohorts, suggesting a lingering cardiovascular vulnerability. Yet despite these compelling epidemiological links, FMD lacks standardized thresholds and reproducibility needed to serve as a routine prenatal screening tool.

A similar distinction between research insights and clinical utility applies to cardiac rehabilitation [66,67] and neurology. While structured physical training has been shown to systematically improve FMD averages in chronic heart failure cohorts—demonstrating the plasticity of the endothelium—these measurements function primarily as surrogate research endpoints [68]. In neuro-angiology, the hypothesis that peripheral endothelial reactivity reflects intracranial vascular health has driven studies in ischemic stroke and cognitive decline. Lower admission FMD values have been associated with early neurological worsening within 72 h of an ischemic stroke [69], and diminished FMD ratios have been observed in patients with Alzheimer's disease and vascular dementia [70], sometimes correlating with Mini-Mental State Examination (MMSE) scores [71]. While these findings offer fascinating insights into the systemic nature of vascular dementia, FMD cannot currently be generalized as a validated biomarker for diagnosing mild cognitive impairment or predicting neuropsychological outcomes in everyday neurological practice [72,73].

4.3. Limitations

Integrating the FMD procedure into standard diagnostic practice presents several significant challenges related to instrumentation, technique, and biological factors [9]. These challenges increase the susceptibility of the indicators to confounding variables. These variables include high operator dependence, the risk of errors when manually stabilizing the ultrasound transducer, and greater relative measurement error as blood vessel diameter decreases.

Additionally, it is crucial to consider natural variability in diurnal blood pressure patterns and autonomic nervous system activity, as well as the time since the last meal. Postprandial lipemia (increased blood fat after eating) and hyperglycemia (elevated blood glucose levels) can contribute to oxidative stress, which inactivates nitric oxide.

Vascular reactivity is also affected by several demographic and physiological factors. These include gender, the phase of the menstrual cycle in women (especially the influence of high estrogen levels during the follicular phase), and menopausal status.

Moreover, sudden vasoconstriction and endothelial toxicity can result from active or passive smoking, recent alcohol or caffeine use, and episodes of acute psychological stress or physical exertion.

The mathematical overestimation of FMD percentage in individuals with a smaller baseline vessel diameter (such as women) complicates direct comparisons across individuals.

The loss of mechanical elasticity in blood vessels, caused by advanced atherosclerosis or medial calcification (also known as Mönckeberg's sclerosis), masks the endothelium's true biochemical reactivity. This skews results in older individuals because stiffened and remodeled arterial walls cannot dilate effectively. As a result, the diagnostic accuracy of the classic FMD test is reduced in this population.

Barriers to Mass Clinical Implementation

The main obstacles to using flow-mediated dilation in clinical practice are its high operator dependence and technical challenges. The FMD procedure requires a specialized ultrasound-trained person. Unlike intima–media thickness measurement, which can be performed on a static image, FMD requires the physician to hold the ultrasound transducer at the same angle over a moving, pulsating artery for several consecutive minutes. Even slight accidental movements of the patient's arm or the clinician's hand can distort the image

and invalidate precise measurements of changes in the artery of less than a millimeter. The FMD test takes 20–30 min, making it impractical for mass screening in busy outpatient clinics. Furthermore, the protocol requires a mandatory rest phase in a quiet room, followed by exactly five minutes of forearm occlusion using a cuff and real-time video recording during the reactive hyperemia phase.

Endothelial function is highly sensitive to external stimuli, necessitating an exceptionally rigorous preparation protocol that is difficult to enforce in mass screening settings. In everyday medical practice, ensuring full patient compliance with these strict dietary and lifestyle restrictions poses a significant logistical challenge. FMD results can vary significantly depending on factors such as room temperature, the patient's stress level, age, gender, and even the current phase of the menstrual cycle.

Accurate FMD calculation requires real-time edge-tracking software that automatically measures arterial wall diameter on a frame-by-frame basis.

The manual measurement of peak arterial dilation on a standard ultrasound machine is subject to significant human error and is impractical for high-volume mass screenings.

The financial implications of equipping every local clinic with advanced, automated edge detection software are a significant barrier to widespread adoption.

5. Conclusions

Flow-mediated dilation (FMD) is the noninvasive gold standard for assessing endothelial function in clinical trials. Its routine clinical use in risk stratification for individual patients is limited. This is due to significant methodological variability and the lack of universally standardized cutoff values.

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Abbreviations

The following abbreviations are used in this manuscript:

FMD	flow-mediated dilation
AGEs	Advanced Glycation End-products
RAGE	Receptor for Advanced Glycation End-products
ICAM-1	Intercellular Adhesion Molecule-1
VCAM-1	Vascular Cell Adhesion Molecule-1
LDL	Low-Density Lipoprotein
PAI-1	Plasminogen Activator Inhibitor-1
NO	nitric oxide
eNOS	Endothelial Nitric Oxide Synthase
PI3K/Akt Pathway	Phosphoinositide 3-kinase/Protein Kinase B
MHz	megahertz
mmHg	millimeter of mercury
PCSK9	Proprotein Convertase Subtilisin/Kexin type 9
PFKFB3	6-Phosphofructo-2-Kinase/Fructose-2,6-Biphosphatase 3
HIF-1α	Hypoxia-Inducible Factor 1-alpha
miRNA	MicroRNA; e.g., miR-21, miR-126
ROS	Reactive Oxygen Species
BH4	Tetrahydrobiopterin

ADMA	Asymmetric Dimethylarginine
Nitric Oxide (NO)o $ONOO^-$	Peroxynitrite
VSMC	Vascular Smooth Muscle Cells
sGC	Soluble Guanylyl Cyclase
cGMP	Cyclic Guanosine Monophosphate
Ca^{2+}	Calcium Ion
MLCP	Myosin Light Chain Phosphatase
IMT	intima–media thickness
PWV	pulse wave velocity

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