MAYO CLINIC

REVIEW

Rethinking Endothelial Dysfunction as a Crucial Target in Fighting Heart Failure

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Abstract

Endothelial dysfunction is characterized by nitric oxide dysregulation and an altered redox state. Oxidative stress and inflammatory markers prevail, thus promoting atherogenesis and hypertension, important risk factors for the development and progression of heart failure. There has been a reemerging interest in the role that endothelial dysfunction plays in the failing circulation. Accordingly, patients with heart failure are being clinically assessed for endothelial dysfunction via various methods, including flow-mediated vasodilation, peripheral arterial tonometry, quantification of circulating endothelial progenitor cells, and early and late endothelial progenitor cell outgrowth measurements. Although the mechanisms underlying endothelial dysfunction are intimately related to cardiovascular disease and heart failure, it remains unclear whether targeting endothelial dysfunction is a feasible strategy for ameliorating heart failure progression. This review focuses on the pathophysiology of endothelial dysfunction, the mechanisms linking endothelial dysfunction and heart failure, and the various diagnostic methods currently used to measure endothelial function, ultimately highlighting the therapeutic implications of targeting endothelial dysfunction for the treatment of heart failure.

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Heart failure (HF) incidence is increasing worldwide as risk factors such as coronary artery disease (CAD), hypertension, diabetes, obesity, and lack of physical activity remain a pervasive problem.[1] Endothelial dysfunction plays an important role in the development of HF.[2,3] It is characterized by nitric oxide (NO) dysregulation, inflammation, and oxidative stress, which impair the capacity of the vascular endothelium to perform its many functions, such as regulation of vascular tone via smooth muscle relaxation, antifibrinolysis, the clotting cascade, and inflammatory processes. All of these mechanisms play a key role in cardiovascular diseases (CVDs) and notably HF pathophysiology (Figure 1). Endothelial dysfunction was a large area of focus during the 1990s and early 2000s and has resurfaced as an emerging area of interest as a potential therapeutic target in patients with HF. In recent clinical trials, results suggest that improving endothelial function in patients with HF concordantly improves heart biomechanics.[4,5]

As clinical trials progress, our understanding of the pathophysiology of endothelial function in patients with HF, as well as our understanding of different diagnostic methodologies, needs to be further elucidated and validated. In this review, we discuss the current understanding of the pathophysiology and molecular mechanisms of endothelial dysfunction in patients with HF. Additionally, we review the different methods currently available to assess endothelial function, highlighting the advantages and disadvantages of each. Lastly, we review relevant clinical trials and the implications of targeting endothelial dysfunction in this patient population.

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THE ROLE OF THE ENDOTHELIUM

The endothelium is comprised of a monolayer of endothelial cells that acts as a semiselective barrier between the bloodstream and the wall of the blood vessels and modulates blood flow, blood clotting, inflammation, and plasma permeability. The vascular endothelium regulates vasomotor tone, clotting factors, and inflammation.[3] More specifically, the healthy vascular endothelium regulates vascular tone by balancing the production of vasodilators and vasoconstrictors, which affect smooth muscle dilation. Nitric

ARTICLE HIGHLIGHTS

- The pathophysiology of endothelial dysfunction plays a significant role in the development and progression of heart failure and cardiovascular disease.
- There are numerous methods for measuring endothelial function, most notably being intracoronary arterial infusions combined with quantitative coronary angiography and Doppler imaging, flow-mediated vasodilation, peripheral arterial tonometry, endothelial progenitor cell-colony forming unit assay, and circulating endothelial progenitor cells, each with distinct advantages and disadvantages.
- Pharmacologic and nonpharmacologic therapies are under clinical investigation for targeting endothelial dysfunction as a means to improve heart failure and cardiovascular disease outcomes.

oxide is the predominant mediator of vasodilatation, but other endogenous factors such as bradykinin, acetylcholine (Ach), catecholamines, endothelium-derived hyperpolarizing factor (EDHF), and prostacyclin also play a role. In fact, emerging research suggests that diminished EDHF-mediated vasodilation is a major contributor to endothelial dysfunction, since EDHF is crucial for small resistance artery relaxation, especially in the setting of high cholesterol or diabetes.[6-8] Furthermore, the endothelium produces antiproliferative and anti-inflammatory cytokines as well as regulates the coagulation cascade through a balanced production of anticoagulant and procoagulant factors. The function of the endothelium is impaired in patients with cardiovascular risk factors or CVD, and this is associated with increased oxidative stress and impaired NO balance, which contribute to the progression of the disease.

Molecular Mechanisms: The Role of NO Signaling

Because NO is fundamental to maintaining vascular homeostasis, its dysregulation is strongly implicated in endothelial dysfunction. Nitric oxide is synthesized when endothelial NO synthase (eNOS) converts L-arginine to L-citrulline. Once produced,

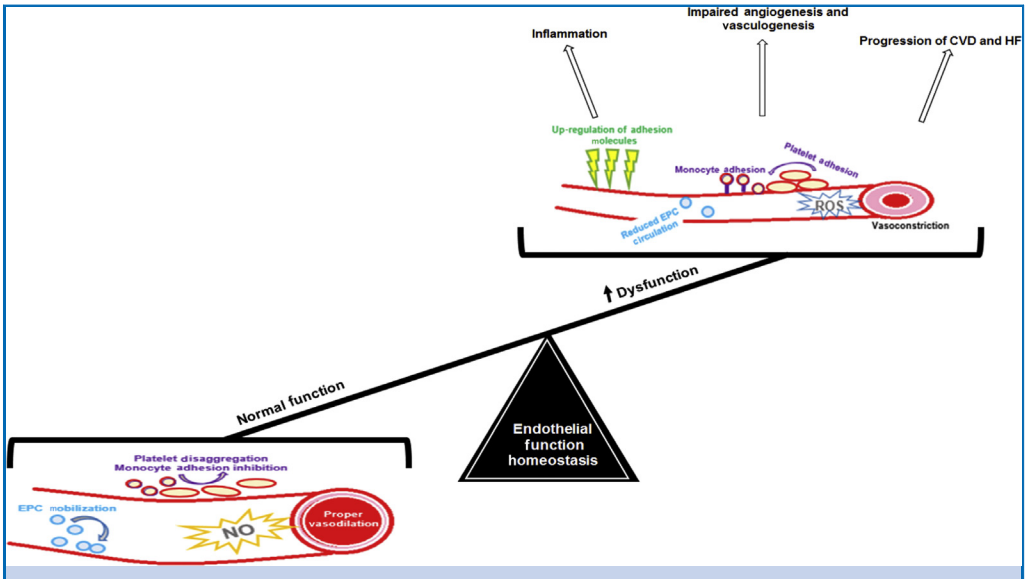

FIGURE 1. The critical balance between endothelial function and dysfunction. When the endothelium is functioning properly, there is proper endothelial progenitor cell (EPC) mobilization, platelet aggregation and monocyte adhesion are inhibited, and nitric oxide (NO) stimulates proper vasodilation. However, when the balance is tipped upward and endothelial dysfunction is high, there is an up-regulation of adhesion molecules and therefore monocyte and platelet adhesion, reduced EPC circulation, and improper NO signaling leading to the production of reactive oxygen species (ROS) and the constriction of the endothelium. Ultimately, this endothelial dysfunction pathology causes systemic inflammation, impaired angiogenesis, and impaired vasculogenesis and contributes to the progression of cardiovascular disease (CVD) and heart failure (HF).

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NO activates soluble guanylyl cyclase and thereby generates cyclic guanosine monophosphate (cGMP) to produce multiple downstream effects. The effects of NO are also mediated via the S-nitrosylation, a posttranslational modification, of effector proteins.[9] Under normal conditions, NO is released from the endothelium and stimulates the relaxation of smooth muscle cells. This release of NO is most notably triggered by shear stress induced by blood flowing over the surface of the vessel; however, NO release can also be stimulated by agonists and vasoactive mediators (eg, Ach, histamine, thrombin, serotonin, adenosine disphosphate, bradykinin, norepinephrine, substance P, and isoproterenol, among others).[10] Moreover, NO has anti-inflammatory (inhibiting leukocyte adhesion and infiltration), antithrombotic (increasing cGMP in platelets), and antioxidant (scavenging free radicals) effects.[10,11]

With regard to the heart, physiologic NO signaling participates in myocardial contractility and relaxation as well as mitochondrial respiration.[12] These effects are due to interactions between NO and Ca^{2+}-handling proteins, including the L-type Ca^{2+} channel, ryanodine receptor/Ca^{2+}-release channel, and sarcoendoplasmic reticulum Ca^{2+} ATPase, the contractile myofilaments, and respiratory complexes. Importantly, NO signaling is regulated by its site of production, which is determined by the spatial localization of the NO synthase (NOS) enzymes.[13] Nitric oxide modulates calcium signaling through S-nytrosylation of calcium channels, thereby regulating excitation-contraction coupling.[9,14-16] In the setting of myocardial infarction or HF, the spatial localization of the NOS enzymes is disrupted, leading to impaired myocardial NO signaling. For instance, myocardial NO has been found to inhibit the positive inotropic responses to β-adrenergic receptor stimulation[17] and further impair the failing heart,[18] thus highlighting the key physiologic and pathophysiologic role NO signaling has in cardiac function.

Molecular Mechanisms: Reactive Oxygen Species and Inflammation

Increased reactive oxygen species (ROS) and inflammation, combined with diminished NO production and bioavailability, are the principal mechanisms underlying the pathophysiology of endothelial dysfunction.[19] The ROS are highly reactive chemical species that are formed both physiologically as a natural by-product of oxygen metabolism, as well as pathologically during environmental and oxidative stress. The ROS can be generated during normal cellular metabolism, as intermediates during redox reactions, or enzymatically by uncoupled eNOS, xanthine oxidoreductase, mitochondrial enzymes, and nicotinamide adenine dinucleotide phosphate oxidase.[20] The uncoupled state of eNOS refers to the dissociation of the ferrous-dioxygen complex, which is when the ROS superoxide anion is generated from the oxygenase domain.[21] Superoxide anion is the primary ROS in the endothelium, which when generated, signals the contraction of vascular smooth muscle and detrimentally scavenges NO within the vascular wall.[22] When superoxide anion reacts with NO by a diffusion-limited reaction, it forms a much more powerful oxidant called peroxynitrite.[23] Most notably, peroxynitrite oxidizes proteins involved in contractility and ion channels, which inhibit mitochondrial energy production. Another important ROS is hydrogen peroxide (H_2O_2), an uncharged, stable, nonpolar ROS that has a longer half-life compared to superoxide and the ability to readily diffuse between cells.[24] Physiologically, H_2O_2 is an important intracellular messenger that regulates endothelial cell growth and proliferation, vasorelaxation, vascular remodeling, and inflammatory responses.[25] However, excessive H_2O_2 is implicated in the alteration of vascular function, induction of vascular smooth muscle cell inflammation and calcification, and the progression of CVD.[26]

At low concentrations, ROS are beneficial and physiologic, contributing to host defense mechanisms against infectious agents, regulating cell growth, cell adhesion, cell senescence, and cellular apoptosis.[20] However, overproduction of ROS has deleterious effects, causing oxidative stress, DNA and cellular damage, vascular cell damage—notably to endothelial cells and vascular smooth muscle cells, ultimately inhibiting proper lipid and protein function—and contributing to systemic inflammation.[27] Elevated levels of ROS are damaging to endothelial function, largely due to disruption of the balance of NO signaling. Oxidants stimulate inflammatory cytokines to further

induce endothelial dysfunction. Inflammatory activation of endothelial cells causes increased expression of selectins, adhesion molecules, and monocyte activation.[28] Ultimately, higher levels of ROS cause systemic inflammation, which can in turn lead to endothelial dysfunction and worsening of CVD.[29] If not addressed, endothelial dysfunction has severe implications for the heart and vascular system.

ENDOTHELIAL DYSFUNCTION AND HF

Endothelial dysfunction is implicated in HF, and the mechanistic relationship between them remains an important subject of investigation. Heart failure is a complex syndrome that results from structural and/or functional impairment of ventricular filling (diastolic dysfunction) or ejection of blood (systolic dysfunction), known as HF with preserved ejection fraction (HFpEF) and HF with reduced ejection fraction (HFrEF), respectively. Heart failure with preserved ejection fraction, characterized by a left ventricular ejection fraction (LVEF) greater than 50%, is an emerging epidemic and thought to represent up to 50% of patients with HF.[30] Importantly, the recommended HF medications (eg, β-blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, angiotensin-receptor-neprilysin inhibitors, and aldosterone antagonists) target HFrEF, or LVEF of 40% or less, and have yielded disappointing results in patients with HFpEF. These recommended medications, or guideline-directed medical therapy, improve morbidity and mortality in patients with HFrEF, whereas guidelines recommend diuretics and aldosterone antagonists in selected patients to ameliorate symptoms and control blood pressure in HFpEF pending future clinical trials.[31,32]

Heart failure with reduced ejection fraction develops as a result of cardiac injury, which can be ischemic from a myocardial infarction or nonischemic (genetic or acquired), leading to systolic dysfunction and eccentric hypertrophy. The standard of care HF medications are antagonists of the renin-angiotensin-aldosterone and adrenergic systems, which help attenuate direct myocardial injury, cell necrosis, and apoptosis. Blunting the neuroendocrine system allows for reverse remodeling of the left ventricle, leading to improvements in LVEF and clinical outcomes. Because HFrEF is a progressive disease, without attenuation of the neuroendocrine system and reverse remodeling, systolic function will continue to decline, leading to advanced HF. Heart failure with reduced ejection fraction can promote endothelial dysfunction via neurohormonal activation, altered shear stress, increased oxidative stress, and a decrease in the production of NO.[2,3] Ultimately, HFrEF fosters an altered redox state in which oxidative stress and inflammatory markers prevail. With this imbalance of NO and oxidative stress (nitroso-redox imbalance),[33,34] there is a subsequent decrease in coronary endothelium-dependent vasodilator capacity, which impairs myocardial perfusion, reduces coronary flow, and worsens ventricular function.[3] These processes culminate in reduced NO bioavailability and worsening endothelial dysfunction, which in turn propagates progression of chronic HFrEF.[3]

In HFpEF, there is no direct cardiac injury, but instead a multitude of comorbidities that may underlie the etiology (eg, hypertension, obesity, diabetes, chronic kidney disease, chronic obstructive pulmonary disease, anemia, and iron deficiency).[35] It has been well documented that patients with HFpEF have impaired endothelial function.[36-38] In this inflammatory state, there is transformation of endothelial cells into fibroblasts, also known as epithelial-mesenchymal transition.[39] The inflammatory state seen with these comorbidities can also cause decreased NO bioavailability leading to decreased cGMP, altered phosphorylation of titin, and microvascular ischemia.[35] As previously discussed, NO modulates the activity of calcium by increasing contractile calcium responsiveness and thereby regulates excitation-contraction coupling.[14] With endothelial dysfunction and decreased NO bioavailability, in combination with increased ROS such as peroxynitrite, there are lower levels of intracellular cGMP and protein kinase G activity—kinases that regulate smooth muscle relaxation, platelet function, cell division, and nucleic acid synthesis.[23] The reduction in cGMP and protein kinase G activity results in increased diastolic cytosolic calcium and thus delayed myocardial relaxation.

Nitric oxide imbalance also affects endothelial progenitor cells (EPCs), impairing endothelial repair and regeneration as well as altering matrix metalloproteinases, which affects cell migration, cardiac hypertrophy, and

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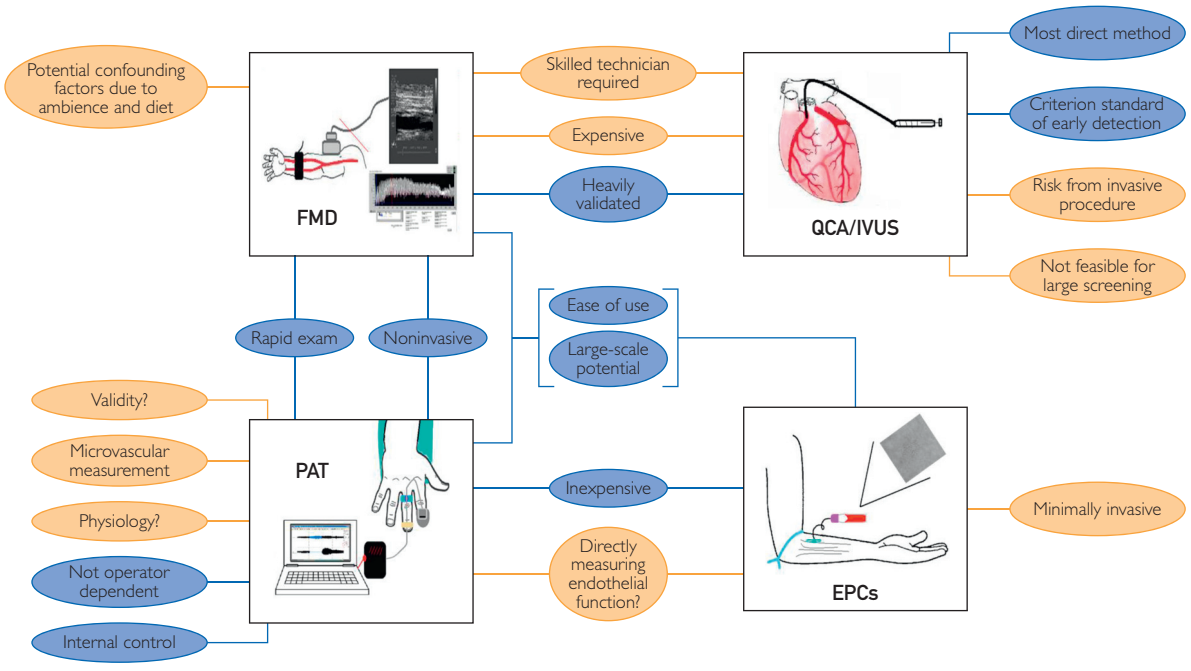

FIGURE 2. The advantages (green) and disadvantages (red) of flow-mediated vasodilation (FMD), quantitative coronary angiography (QCA)/intravascular ultrasound (IVUS), peripheral arterial tonometry (PAT), and endothelial progenitor cells (EPCs) techniques. exam = examination.

atherosclerotic plaque stability. Additionally, alterations in matrix metalloproteinases can cause excess production and accumulation of extracellular matrix structural proteins, leading to fibrosis that results in enhanced myocardial stiffness.[40] Also, endothelial dysfunction allows for transmigration of monocytes into the myocardium, leading to interstitial fibrosis causing diastolic dysfunction.[41] All of these mechanisms can lead to concentric left ventricular hypertrophy, a main contributor to HFpEF. Because current standard of care HF treatments are less effective for HFpEF, evidenced by the results from phase 3 clinical trials—I-PRESERVE (Irbesartan in Patients with Heart Failure and Preserved Ejection Fraction),[42] PEP-CHF (Perindopril in Elderly People with Chronic Heart Failure),[43] TOPCAT (Treatment of Preserved Cardiac Function Heart Failure With an Aldosterone Antagonist),[44] and CHARM-Preserved (Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity)[45]—novel approaches need to be explored that target endothelial dysfunction in order to ameliorate HFpEF and prevent adverse CVD outcomes.

METHODS FOR MEASURING ENDOTHELIAL FUNCTION

There are numerous methods for measuring endothelial function, most notably intracoronary arterial infusions combined with quantitative coronary angiography (QCA) and Doppler imaging, flow-mediated vasodilation (FMD), peripheral arterial tonometry (PAT), EPC—colony-forming unit (EPC-CFU) assay, and circulating EPCs (Figure 2). Intracoronary Ach infusion was the first method discovered for measuring endothelial function; however, due to its invasive nature, FMD has largely replaced ACh as the most widely used method. PAT is a newly emerging noninvasive method that is currently under investigation as a supplement to FMD. Lastly, EPC measurements are generally considered a less direct method of measuring dysfunction, but studies have shown their important prognostic role in CVD.[46]

Intracoronary Artery Infusions Using ACh and Other Vasoactive Substances

In 1986, QCA combined with Ach infusions was the first method utilized to detect endothelial

function and is considered the criterion standard for early detection of dysfunction.[47] Intracoronary arterial infusion is the most direct, yet most invasive and expensive, method of measuring endothelial function. During QCA, a contrast agent is injected into the coronary artery and vessel diameter is measured from multiple planes of view. After obtaining baseline measurements, either ACh or other vasoactive substances (ie, substance P, bradykinin, serotonin) are infused into the artery—typically as multiple doses infused for 1 to 2 minutes—and coronary blood flow is continuously measured using Doppler techniques. Instead of QCA, intravascular ultrasound (IVUS) can also be employed along with Ach or other vasoactive infusions to measure coronary blood flow velocity using a Doppler-guided wire to obtain vessel measurements. Regardless of QCA or IVUS, endothelial function is then calculated by determining volumetric coronary blood flow and evaluating it on a dose-response curve.[48] In a healthy endothelium, Ach causes the coronary arteries to dilate. However, in a patient with endothelial dysfunction, Ach instead causes a paradoxical vasoconstriction of the arteries.[47]

Quantitative coronary angiography and IVUS are both widely validated techniques that have documented utility in studying endothelial function in patients with CVD and HF.[49-51] Schächinger et al[49] found that coronary endothelial vasodilation dysfunction predicted long-term (median 7.7-year follow-up in 147 patients) atherosclerotic disease progression and risk of cardiovascular events. Reriani et al[52] recently reported that coronary endothelial function can be an invaluable tool to reclassify risk stratification of patients with early CAD, highlighting its superior discrimination over standard methods such as Framingham Risk Score. However, due to the invasiveness of the method combined with the high risk associated with coronary catheterization, intracoronary studies are not feasible for screening large populations. Consequently, alternatives to this invasive method emerged in the 1990s, and numerous studies since then have affirmed similar results between using coronary artery response and brachial FMD.[53]

Flow-Mediated Vasodilation

In 1992, FMD was first introduced as a noninvasive method of measuring endothelial dysfunction and has gained popularity as the most robust and widely validated technique.[54] To measure FMD, the patient is positioned supine and a blood pressure cuff is placed above the antecubital fossa. After a baseline image of the brachial radial artery is obtained, the cuff is inflated to approximately 50 mm Hg above systolic pressure in order to occlude arterial inflow for a short period. Subsequently, the cuff is deflated, which creates a potent shear stress enabling the induced reactive hyperemia to dilate the brachial artery. This shear stress is mediated by NO released from endothelial cells along with other vasoactive mediators. The change in diameter of the artery is continuously recorded for up to 2 minutes after the cuff deflation via ultrasound, and the FMD is reported as a percent change in poststimulus diameter compared to the baseline diameter.

Flow-mediated vasodilation has gained popularity in clinical trials because of its ease of use, efficiency, and noninvasive nature. However, various factors including food intake, coffee intake, ambient temperature, drugs, vitamins, exercise, tobacco use, and menstrual cycle can affect a patient's FMD, potentially confounding study results. Therefore, it is essential that patients fast before the assessment and abstain from taking medicines, vitamins, exercising, and using tobacco the day of the test. Furthermore, it is important that the study room conditions are held constant throughout the trial, meaning a consistent, comfortable room temperature, same lighting, and minimal environmental stress. Despite these potential limitations, Ghiadoni et al[55] performed a nationwide, multicenter study and found that standardized and rigorous methodology among centers resulted in accurate and homogeneous results, highlighting the reproducibility of FMD assessments.

Abundant cardiovascular studies using FMD have generated insight into the role endothelial dysfunction plays in CVD and HF. Flow-mediated vasodilation has been reported to be significantly impaired in patients with atherosclerosis, hypertension, dilated cardiomyopathy, ischemic cardiomyopathy, CAD, peripheral artery disease, and congestive HF.[56,57] Furthermore, studies utilizing FMD have found that it serves as an important prognostic tool for CVD diagnosis.[57,58] In a cohort of 3026 individuals, Yeboah et al[58] found that FMD was significantly

diminished and inversely associated with incident cardiovascular events, enabling FMD to be used as a means to classify patients into low-, intermediate-, and high-risk categories.

Peripheral Arterial Tone

With the increasing recognition of the role endothelial dysfunction plays in HF and the powerful predictive role proper assessment plays in discerning cardiovascular events, there is a necessity for novel noninvasive techniques. This need led the way for the emergence of reactive hyperemia-PAT technology. In the early 2000s, the idea of measuring peripheral microvascular endothelial dysfunction surfaced, and EndoPAT—a device developed by Itamar Medical Ltd—was introduced as a more rapid, cheaper, and easier method for assessing endothelial function, relying on peripheral arterial circulation in the finger. Similar to brachial FMD, hyperemia is induced by occluding blood flow through the brachial artery utilizing a cuff for 5 minutes and measuring the arterial tone changes in peripheral arterial beds generally in the index finger. On release of the cuff, reactive hyperemia is automatically calculated by the device as a postocclusion to preocclusion signal ratio, therefore making it non—operator dependent. In addition, a baseline measurement is obtained in the other arm, serving as an internal control.

One of the major drawbacks of PAT currently is that the physiology remains unclear. PAT measures microvessel dilatation, as opposed to FMD that measures macrovascular dilatation, and is thus only partly dependent on NO. Notably, inhibiting NO and subsequently measuring endothelial function via PAT attenuates the response by less than 50%, whereas using FMD nearly completely inhibits the response.[59] Because PAT is a newer technology and the goal of both technologies is to measure endothelial function, many studies have tried to prove PAT reliability by validating the correlation between FMD and PAT.[60] However, studies have yielded mixed results in this comparison. For example, both Hamburg et al[61] and Lee et al[62] found that there was no correlation between PAT and FMD, questioning the reliability of PAT. Conversely, Kuvin et al[63] found a significant correlation between reactive hyperemia measured by FMD and PAT. Irrespective of the variable results comparing FMD and PAT, PAT has been reported to be a suitable diagnostic tool for patients with HF.[64,65]

EPC and Hematopoietic and Endothelial Lineage Assessment Methodologies

Endothelial progenitor cells are vital to proper endothelial function, playing a pivotal role in maintaining vascular homeostasis and mediating vascular repair in damaged endothelium by stimulating the release of NO from the endothelium as well as by incorporating into old and damaged endothelium. Endothelial progenitor cells can be measured via flow cytometry as well as by cell culture techniques. When measuring EPCs via cell culture methodologies, there are 2 different populations of EPCs, early and late outgrowth. Recently, there has been a large debate in the literature regarding proper nomenclature, lineage, and function. Early outgrowth EPCs, identified by the classic EPC-CFU method of Hill et al,[66] are now being considered myeloid angiogenic cells and circulating angiogenic cells, which have a strong paracrine ability that stimulates angiogenesis and enhances endothelial tube network formation.[67] Conversely, late outgrowth EPCs are now identified as endothelial colony-forming cells (ECFCs), outgrowth endothelial cells, and blood outgrowth endothelial cells, which have an intrinsic tube-forming capacity and actively participate and incorporate into new blood vessel formation and vascular repair.[67] Both early and late outgrowth EPCs have been found to be prognostic of endothelial dysfunction and furthermore correlate with CVD and HF.[68,69] Accordingly, assays for both early and late EPCs can be used to diagnose endothelial dysfunction.

To measure early outgrowth EPC-CFUs, a blood sample is obtained from the patient and the mononuclear cell layer is isolated on a Ficoll-Paque (GE Healthcare Life Sciences) density gradient. After multiple washes, the cells are plated on fibronectin-coated dishes. Two days later, these cells are replated onto smaller dishes, and on day 5, EPC-CFUs are counted. This technique is straightforward, minimally invasive, and cost effective. However, it is heavily operator dependent—and therefore colony reads can vary across investigator—and furthermore, the link between EPC-CFUs and

endothelial function is less clear than with FMD. However, low EPC-CFUs have been found to be hallmarks of endothelial dysfunction, and numerous studies have reported that EPC-CFUs are inversely associated with cardiovascular events and disease progression.[46,56] Hill et al[46] conducted a landmark study in which they documented that EPC-CFUs were a better predictor of endothelial function than conventional risk factors, and furthermore, EPC-CFUs are a potential biomarker for vascular function and increasing cardiovascular risk. Along this line, our laboratory specifically documented that patients with dilated cardiomyopathy and endothelial dysfunction had severely impaired EPC-CFUs, and allogeneic mesenchymal stem cell treatment restored these numbers.[56]

Late outgrowth EPCs, or ECFCs, are also obtained from the blood by isolating the mononuclear cell layer on a Ficoll-Paque density gradient. However, these cells are plated on collagen using endothelial growth medium and can be cultured for weeks as well as passaged for expansion. Colonies are assessed anywhere from 5 to 26 days after being plated. Like EPC-CFUs, ECFCs have also been found to be prognostic of CVD[70] and mortality in HF.[71] Additionally, this technique is also minimally invasive and cost effective, and cells can be expanded and utilized for further studies.

Alternatively, circulating EPCs can be enumerated using flow cytometry, specifically staining for CD133, CD34, and KDR. However, this method has produced mixed results, potentially because high circulating EPC number does not ascertain that these cells are functionally contributing to endothelial function. Endothelial progenitor cells are a heterogeneous population, and accordingly, measuring EPC-CFUs and ECFCs appears to assess endothelial dysfunction more accurately.[66,72]

CLINICAL TRIALS AND ENDOTHELIAL FUNCTION

Case-control studies have clearly documented a link between endothelial dysfunction and the progression of CVD and HF, highlighting how endothelial dysfunction can be a vital prognostic tool for evaluating CVD stages.[49,54] These correlations have contributed to the hypothesis that targeting endothelial dysfunction could lead to the amelioration of CVD. In recent clinical trials, results have suggested that improving endothelial function in patients with HF may concordantly improve cardiac biomechanics.[5,73] Recently, we found that allogeneic mesenchymal stem cell treatment significantly improved endothelial function in patients with HF due to nonischemic dilated cardiomyopathy.[5,56] Mesenchymal stem cell treatment also resulted in increased ejection fraction, 6-minute walk distance test results, and Minnesota Living with Heart Failure questionnaire scores, along with tumor necrosis factor α reduction.[5,55] Our study utilized FMD and EPC-CFU assays to assess endothelial function.

Exercise has been reported to increase blood flow and shear stress, therefore increasing endothelial NOS activity and NO production and reducing inflammation, which are direct components of endothelial function,[74] making it an appealing candidate for improving endothelial function. A growing number of clinical trials are investigating the effect of exercise on endothelial function in patients with HF and HFpEF.[75] In a 458 patient meta-analysis, Pearson and Smart[76] revealed that aerobic exercise significantly improves FMD in patients with HF, suggesting that improving endothelial function via cardiac rehabilitation may be an important therapy for patients with HF. Indeed, Tanaka et al[77] recently reported that cardiac rehabilitation improved prognosis in patients with HF, although with a greater effect on patients with HFrEF than HFpEF, and this improvement was attributed to the impact on improving endothelial function. Ozasa et al[78] utilized EndoPAT to demonstrate that machine-assisted cycling and conventional exercise training improved endothelial function and 6-minute walk distance in 27 patients with HF. Erbs et al[79] found that bicycle training for 12 weeks in patients with advanced chronic HF improved FMD and maximum oxygen consumption and notably increased ejection fraction by approximately 10%. Sandri et al[80] assessed EPC numbers and function and found that 4 weeks of exercise was efficacious in improving endothelial function in 60 patients with chronic HF. Conversely, Angadi et al[81] investigated the effect of high-intensity interval training vs moderate-intensity aerobic continuous training in patients with HFpEF and

found that although peak oxygen consumption improved, FMD did not. Kitzman et al[82] evaluated the effect of endurance training on FMD and carotid artery stiffness in 63 patients with HFpEF and found results similar to those of Dall et al,[83] with improvement in peak oxygen consumption but no change in either endothelial function via FMD or arterial stiffness. Nevertheless, current guidelines strongly recommend appropriate amounts of exercise for primary and secondary prevention of CVD and HF.[84,85]

Two statin clinical trials yielded encouraging results, reporting that statins improve endothelial function and hemodynamics in patients with HF, CAD, and atherosclerosis.[86-88] Antoniades et al[88] found that statins improve endothelial function, assessed by FMD, in patients with CAD. Additionally, they reported a potential mechanism for this improvement, namely, an increase in NO bioavailability caused by a reduction in uncoupled endothelial NOS-derived superoxide and an increase in vascular BH4 bioavailability via the up-regulation of guanosine triphosphate cyclohydrolase 1. The same group later demonstrated that 4 weeks of atorvastatin treatment significantly improved endothelial function (measured by FMD and endothelial-independent vasodilation) and EPC mobilization and reduced tumor necrosis factor α in patients with ischemic HF.[86] Erbs et al[87] found that high-dose rosuvastatin in 42 patients with HF resulted in a dramatic improvement in FMD, LVEF, vascular endothelial growth factor, circulating EPCs, and capillary density and that these effects were accompanied by decreased oxidized low-density lipoprotein.

Blood pressure medications have also been explored, yielding mixed results. Specifically, nebivolol, a β_1-selective receptor blocker with NO-potentiating effects, demonstrated efficacy in improving endothelial function.[89,90] Conversely, metoprolol, a β_1-selective receptor blocker, and spironolactone, an aldosterone antagonist, had minimal to no effects on endothelial function.[91,92]

There are abundant ongoing clinical trials assessing endothelial function in patients with CVD. Albumin, nutraceutical therapies, new oral anticoagulants, sildenafil, beetroot juice, tetrahydrobiopterin (BH4), sitagliptin, bioresorbable stents, remote ischemic preconditioning, and yoga are interventions currently under investigation for improving endothelial function. More specifically, the Endothelial Function After Cardiac Surgery study (ClinicalTrials.gov Identifier: NCT02882074) is evaluating whether administration of albumin during cardiac surgery improves postoperative endothelial function compared to hydroxyethyl starch solutions using EndoPAT. In the NUTRENDO (Effects of Neutraceutical Therapies on Endothelial Function, Platelet Aggregation, and Coronary Flow Reserve) trial (ClinicalTrials.gov Identifier: NCT02969070), Trimarco et al are investigating the effects of nutraceutical therapies on endothelial function, platelet aggregation, and coronary flow reserve in patients with hypercholesterolemia, specifically measuring reactive hyperemia index. Kim et al are testing the effect of new oral anticoagulants (dabigatran, rivaroxaban, warfarin) for improving endothelial dysfunction—assessed via EndoPAT—and therefore preventing the progression of atherosclerosis in patients with atrial fibrillation (ClinicalTrials.gov Identifier: NCT02544932). Liang et al are recruiting patients with rheumatoid arthritis to see if sildenafil improves endothelial function, measured by FMD and biomarkers, and is thereby an effective strategy for CVD prevention in patients with rheumatoid arthritis—a patient population associated with a 2-fold risk of CVD (ClinicalTrials.gov Identifier: NCT02908490). The NITRATE-OCT (Investigating the Effects of Dietary Nitrate on Vascular Function, Platelet Reactivity and Restenosis in Stable Angina; ClinicalTrials.gov Identifier: NCT02529189) trial is investigating whether beetroot juice reduces angina symptoms as well as long-term risk of myocardial infarction in patients with CVD, utilizing FMD as an outcome measurement. Alongside this trial, the NITRATE-TOD (Investigation of Dietary Nitrate Effects in Hypertension-induced Target Organ Damage; ClinicalTrials.gov Identifier: NCT03088514) study is assessing beetroot juice in patients with hypertension. In the COPD-LT (Long Term Nitric Oxide Bioavailability on Vascular Health in Chronic Obstructive Pulmonary Disease; ClinicalTrials.gov Identifier: NCT02774226) trial, patients with chronic obstructive pulmonary disease are being treated with BH4 to see if endothelial dysfunction is improved (measured as an increase in FMD and change in biomarkers of oxidative stress)

and therefore future atherosclerotic CVD and events. The SAVORO (Sitagliptin Effects on Arterial Vasculature and Inflammation in Obesity; ClinicalTrials.gov Identifier: NCT02576288) trial is administering sitagliptin in patients with atherosclerosis and inflammation to see if FMD and carotid stiffness improve, hypothesizing that treatment will halt atherosclerosis progression to serious CVD events. Testing the efficacy of bioresorbable and metallic stents in patients treated for stable angina, Gomez-Lara et al (ClinicalTrials.gov Identifier: NCT02738658) are using endothelial dysfunction measured by Ach infusions and QCA as a secondary outcome measurement. The PIXIE (Prevention of Myocardial Injury in Non-cardiac Surgery; ClinicalTrials.gov Identifier: NCT02344797) trial is studying whether remote ischemic preconditioning prevents myocardial injury and infarction, specifically assessing endothelial dysfunction via EndoPAT as an outcome measurement. Hunter et al are exploring the effect of Bikram yoga on arterial stiffness and endothelial dysfunction as measured by FMD (ClinicalTrials.gov Identifier: NCT02488148), hypothesizing that yoga will improve vascular endothelial dysfunction and therefore reduce risk factors for CAD.

In addition to interventional testing, numerous clinical trials are testing the prognostic value of measuring endothelial function in patients with CVD. Bairey Merz et al (ClinicalTrials.gov Identifier: NCT00573027) are studying microvascular disease in HF and cardiac syndrome X, assessing the predictive value of the noninvasive PAT, and furthermore elucidating risk factors and treatment using PAT as a primary outcome and coronary flow measurements with Ach as a secondary outcome. Thomas et al (ClinicalTrials.gov Identifier: NCT02636062) are aiming to define the rate of index atherosclerotic plaque developments and identify new markers of CAD progression in patients with CAD and endothelial dysfunction utilizing EndoPAT combined with blood biomarker assessments. O'Neal et al hypothesized that the pathophysiology of delirium after cardiac surgery is due to the stress caused by systemic inflammation causing endothelial dysfunction, making change in endothelial function their primary end point (ClinicalTrials.gov Identifier: NCT02688179). The Mainz Registry of Flow-Mediated Constriction (ClinicalTrials.gov Identifier: NCT01787370) is investigating the prognostic value of FMD in patients undergoing coronary angiography. The POETRYabd (Perioperative Endothelial Dysfunction in Patients Undergoing Major Acute Abdominal Surgery; ClinicalTrials.gov Identifier: NCT03010969) trial is examining the correlation between perioperative endothelial dysfunction—measured by EndoPAT—and major adverse cardiovascular events as well as noncardiovascular complications. Tibirica et al are evaluating the predictive efficacy of measuring systemic microvascular endothelial dysfunction via microvascular reactivity in patients with infective endocarditis (ClinicalTrials.gov Identifier: NCT02940340).

In summary, a growing number of clinical trials support the notion that drugs targeting endothelial dysfunction in patients with HF or CVD may be a feasible therapeutic option. However, it is notable that many of these interventions have only been tested in one clinical trial with low enrollment numbers. Currently, there are ongoing clinical trials assessing not only various interventions for treating endothelial dysfunction in patients with CVD but also assessing the prognostic value of endothelial dysfunction for CVD progression.

CONCLUSION

Endothelial dysfunction has reemerged as a disease process or biomarker warranting further clinical investigation. As reviewed herein, the pathophysiology of endothelial function plays a significant role in the development and progression of HF and CVD. Methods for studying endothelial function clinically have improved, been validated, and correlate with CVD outcomes, but more effective therapies for targeting endothelial dysfunction in order to ameliorate HF and CVD outcomes are needed. As endothelial function is recognized as a crucial component underlying HF and CVD, we propose that targeting endothelial dysfunction will provide, at least in part, the breakthrough therapy needed.

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Abbreviations and Acronyms: **Ach** = acetylcholine; **CAD** = coronary artery disease; **cGMP** = cyclic guanosine monophosphate; **CVD** = cardiovascular disease; **ECFC** = endothelial colony-forming cell; **EDHF** = endothelium-derived hyperpolarizing factor; **eNOS** = endothelial nitric oxide synthase; **EPC** = endothelial progenitor cell; **EPC-CFU** = EPC—colony-forming unit; **FMD** = flow-mediated vasodilation; **HF** = heart failure; **HFpEF** = HF with preserved ejection fraction; **HFrEF** = HF with reduced ejection fraction; **H_2O_2** = hydrogen peroxide; **IVUS** = intravascular ultrasound; **LVEF** = left ventricular ejection fraction; **NO** = nitric oxide; **NOS** = NO synthase; **PAT** = peripheral arterial tonometry; **QCA** = quantitative coronary angiography; **ROS** = reactive oxygen species

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Potential Competing Interests: Drs Premer and Hare report having a materials and methods patent for endothelial dysfunction. Dr Hare reports having a patent for cardiac cell-based therapy; holds equity in and maintains a professional relationship with Vestion, Inc. as a consultant and member of the Board of Directors and Scientific Advisory Board; is the chief scientific officer, a compensated consultant, and advisory board member for Longeveron LLC, holds equity in Longeveron, and is the coinventor of intellectual property licensed to Longeveron. The other authors report no competing interests.

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