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Review

Flow Mediated Dilatation as a Biomarker in Vascular Surgery Research

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Endothelial dysfunction is one of the hallmarks of atherogenesis, and correlates with many cardiovascular risk factors. One of the features of endothelial dysfunction is the loss of nitric oxide (NO) bioavailability, resulting in derangements in the vasodilatory response of the vessel wall. Flow mediated dilatation (FMD) of the brachial artery is an accepted method for non-invasive assessment of systemic endothelial function. FMD is examined extensively in the context of cardiovascular research, and has been utilised as a routine assessment in large cohorts such as the Framingham Heart Study, Young Finns Study, and Gutenberg Heart Study. However, FMD is less known in the context of vascular surgery research, despite the similarities between the underpinning disease mechanisms. This review will provide a summary of FMD in terms of its history of development and the conduct of the test in research settings. It will further highlight the key literature of FMD as a biomarker for vascular surgeons, particularly in the context of abdominal aortic aneurysms and lower limb peripheral arterial disease.

Key words: Flow mediated dilatation, Biomarkers, Abdominal aortic aneurysm, Peripheral arterial disease

Introduction

The vascular endothelium is the predominant regulator of vascular homeostasis, governing the processes of vasoconstriction and vasodilation, thrombogenesis, fibrinolysis, smooth muscle cell proliferation, and cell adhesion[1]. Calcification, stenosis and atherosclerotic plaques can attenuate the effectiveness of these functions, bringing about endothelial dysfunction in regulatory behaviour and impacting the overall cardiovascular health of an individual[2]. Extensive literature suggests endothelial dysfunction as an early hallmark in the development of atherosclerosis[3], and indeed the ability of endothelial dysfunction to reflect atherosclerotic disease severity, and as a predictor of future cardiovasulcar events[3, 4].

A recognised non-invasive assessment of endothelial function is flow mediated dilatation (FMD) of a main conduit artery, such as the brachial artery. The FMD measurement capitalises on the reaction of the vascular endothelium when subjected to shear stress. The typical response in a healthy individual is an observable dilation[5, 6]. Early studies found low shear stress to be correlated with atherosclerotic plaque burden, resulting in uneven stiffness across various regions of artery[7, 8]. It was subsequently observed that external stimuli, such as compression of the artery from the skin surface, could result in the same provocation of the vasodilatory response that high sheer stress from blood flow induced, resulting in the development of FMD as a measure of responsiveness of the vascular endothelium.

The measurement of FMD has been widely adopted in cardiovascular research, and is reproducible in the setting of clinical trials[9, 10]. FMD has been shown to associate with other markers that are relevant in the context of cardiovascular disease, such as serum levels of docosahexaenoic acid (a polyunsaturated fatty acid)[11]. It has also been examined in the context of paediatric populations who may be at risk of subse-

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quent atherosclerotic disease[12]. Acceptance of FMD as an adjunctive marker for cardiovascular risk prediction is evident by its inclusion in large prospective cohort studies such as the Framingham Heart study[13], Young Finns studies[14], and the Gutenberg Heart Study[15]. In Japan, a population known for its long life expectancy, the measurement of FMD has been adopted into routine clinical practice, where the Government reimburses the fee for FMD tests performed for clinical practice[16].

The aim of this review is to provide a summary of the FMD test in terms of its history of development, conduct and analysis of the test, and its relevance as a potential biomarker in vascular surgery research.

Historic Perspective

The methods of FMD measurement in humans first emerged in the literature in 1989 through the seminal work of Anderson *et al.*[17], and was subsequently applied into the clinical research setting by Celermajer *et al.* in 1992[6]. Although it is feasible to measure FMD in different conduit arteries, such as the radial, brachial, or femoral artery. Brachial arteries are typically preferred due to the ease of the procedure. The brachial artery is relatively superficial and large enough for clear imaging, and enables the application of a blood pressure cuff in the forearm with ease.

During the early phase of adoption of this technique, many variations emerged in the methodology leading to significant repeatability and comparison issues between studies. Emergence of standard timing protocol began the process of standardising the method[18]. Initially, analysis of recorded sonograms was labour intensive and subject largely to observer interpretation. However, at the turn of the millennium the introduction of automated edge detection software offered a faster and more reproducible method of analysis[19]. Subsequently the technique was further standardised to place the occlusion cuff on the forearm which further improved the reproducibility of FMD values were observed between distal and proximal placements on the upper limb[20].

Conduct of the FMD Procedure[18, 21]

The FMD procedure should be performed in a dimly lit quiet room, with the patient in the supine position. ECG tabs can be applied to enable ECG gated acquisition of images. The brachial artery of the right arm is identified by ultrasound and a baseline measurement of lumen diameter obtained. To ensure the stability and fixed position of the ultrasound probe during recording, it is beneficial to hold the ultrasound probe in place using a stereotactic probe holding device. Different variations of the stereotactic fixation device exist. It is simple to incorporate a customised silicon mould of a ultrasound probe holder to a stereotactic clamp with a central magnetic base (UK RS online, Cat# 213-0670). This enables magnetic fixation of the clamp against a metallic table surface which serves as an arm rest.

Supra-systolic pressure is applied to the forearm via a blood pressure cuff for 5 minutes to achieve blood flow occlusion. The diameter of the brachial artery is recorded at specific time points during flow occlusion and after the release of the blood pressure cuff to assess flow mediated changes in diameter. Subsequent computer analysis allows for the calculation of a percentage increase in lumen diameter during FMD.

Off-line analysis of flow mediated diameter changes can be performed using commercially available software, such as Vascular Research Tool (Medical Imaging Applications LLC, USA). The percentage change in flow mediated dilatation or constriction can be calculated by: (Δbrachial artery diameter before and after flow stimuli/brachial artery diameter at baseline).

FMD can be affected by diurnal rhythm, medications, and sympathetic stimuli such as caffeine, nicotine, and exercise. Therefore every attempt should be made to minimise these effects. For example, changes in medications should be recorded and accounted for; participants should abstain from tobacco, coffee and tea for at least 24 hours before the procedure; allow a period of rest in the quite room before undertaking the FMD measurement; perform the test at around the same time if serial measurements are taken from one person. It is also important for each research lab to conduct quality assurance exercises to ensure reproducibility of the analysis.

Next Generation Device for the Measurement of FMD

The conduct of FMD can be challenging. It requires dedicated training of the operator, adherence to standard operating protocols for the acquisition and analysis of the test, and quality assurance exercise to minimise intra-observer variation. Additional hardware modifications (such as a customised stereotactic holder for the ultrasound probe) are also recommended. Slight variations in the conduct of FMD tests can exist between different centers, which means it may be difficult to compare the FMD values between different studies. Despite the extensive literature on FMD in research settings, the lack of a stan-

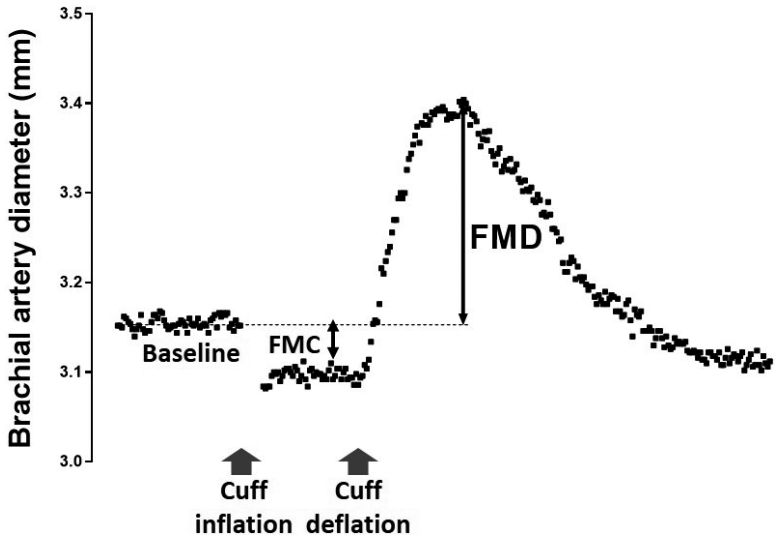

Fig. 1. Graphical illustration of the time course of FMC and FMD

dardised international test procedure affects the ability for FMD to be applied as a clinical test.

A semi-automated device for the FMD test (UNEXEF, UNEX, Nagoya, Japan) overcomes these challenges and has been commercially available in Japan since 2007. The UNEXEF device provides automated vessel wall tracking and real time FMD/FMC analyses, which in term reduces operator related variations. Many investigators in Japan have adopted this device for their investigations on FMD in the context cardiovascular research[22-24]. This device has received FDA approval (K131973) and is currently subject to the CE Mark approval process.

Mechanisms of Flow Mediated Changes in Conduit Artery

Flow Mediated Dilatation

The fundamental principle of FMD is to measure the bioavailability of nitric oxide (NO), the primary vasodilator in the human cardiovascular system[25]. The mechanism for NO-dependent vasodilation is through the enzyme endothelial Nitric Oxide Synthase (eNOS) which facilitates the production of NO from L-Arginine. NO diffuses through the tissue layers to the smooth muscle to initiate the production of cyclic GMP through Guanylate Cyclase, resulting in vasodilation[26].

Nitroglycerine Mediated Dilatation

Nitroglycerine mediated dilatation (NMD) is regularly performed as part of the FMD test as a control procedure[21]. Pharmacological supplementation of NO (via the administration of nitroglycerine) triggers the NO mediated response downstream of its receptor (guanylyl cyclase) within the vascular smooth muscle cells (VSMC). In the presence of normal NO bioavailability, FMD may still be impaired if there is derangement in the VSMC receptor pathway. NMD is therefore often conducted as part of the FMD protocol as a positive control for FMD. For the assessment of NMD, 400 μg of glyceryl trinitrate can be administered sublingually at the end of the FMD protocol, followed by further recording of the brachial artery diameter to record the maximal dilatation achieved.

Flow Mediated Constriction

The process of occluding the brachial artery induces a low flow state, and can lead to constriction of the artery called flow mediated constriction (FMC)[27]. This is thought to reflect the basal vasoconstrictive tone conferred by the release of endothelin[28]. Brachial artery FMC is reported as apparent in only 40% of individuals, but when it does occur it can be associated with both a delayed and blunted FMD response[29].

Time Course of the Flow Mediated Response

The temporal relationship of the flow mediated responses are illustrated in **Fig. 1**. Following baseline vessel lumen diameter measurements (FMB) the occlusion cuff is inflated for a period of five minutes; during which time the vessel may constrict (FMC). FMC is denoted by the minimum arterial diameter observed before the blood pressure cuff deflation (typically taken within 60 seconds of cuff deflation). After cuff deflation, brachial artery lumen diameter is continuously measured. FMD is typically defined by the

maximal diameter recorded during the three minutes after cuff deflation.

FMD as a Biomarker in the Context of Abdominal Aortic Aneurysms

The earliest study to examine FMD as a research test in human AAAs was reported in 2002, by Knipp *et al.*[30]. FMD and NMD were measured in 30 patients with AAA, peripheral arterial disease (PAD), or healthy volunteers (HVs). Subjects with AAA exhibited the worst FMD response among the three groups. A diminished NMD was also observed in the AAA group. Patient with PAD had diminished FMD response compared to HVs, but not in their NMD responses. Around the same time, Gokce *et al.* examined the association between pre-operative FMD and the occurrence of peri-operative complications after major vascular surgery (including 24 patients undergoing AAA repair). They found that patients who suffered postoperative consequences had a lower FMD pre-operatively[31].

Akamatsu *et al.* provided two subsequent reports regarding FMD in AAA patients. The first study examined FMD in a cohort of AAA and PAD patients ($n=30$ and 27 respectively) compared to HVs. HVs had significantly higher FMD than patients with AAA or PAD. No difference in NMD was observed between patients and HVs. (Of note the age of HVs was also significantly younger in this study)[32]. In their other study, FMD and NMD was measured in patients with PAD or AAA/thoracic aortic aneurysm ($n=26$ and 47 respectively). During the follow up period (47 ± 13 months), 18 cardiovascular events were recorded. FMD and NMD were significantly lower in subjects with events. In Cox regression, lower FMD or NMD independently predicted future cardiovascular events[33].

Medina *et al.* was the first to report the correlation between AAA size and FMD[34]. In this cross sectional study of 30 AAA patients (mean AAA diameter 43 mm), an inverse correlation was observed between aneurysm diameter and FMD. They further observed an inverse correlation between FMD and plasma CRP levels, while a positive correlation between AAA diameter and plasma CRP levels was seen. Medina concluded that the increased levels of inflammation have an effect on the bioavailability of NO. Further to these observations, Sung *et al.* examined FMD in 78 subjects (HVs $n=15$, small AAAs $n=27$, or large AAAs $n=36$). The AAA patients demonstrated significantly lower vasodilation than the HVs, as well as a significant difference between the AAA size groups. In addition, fewer circulating endothelial progenitor cells (EPCs) were present in AAA patients than in HVs. EPCs functions (proliferation, adhesion, migration, tube formation etc) were also significantly impaired in AAA patients[35].

The Oxford Abdominal Aortic Aneurysm Study was the first to systemically examine FMD during the progression of AAA in humans. In this largest cohort of AAA patients with FMD measurement ($n=162$), there was a significant inverse correlation between FMD and the diameter of AAAs (median diameter = 50 mm). Eighty-eight of the participants with small to moderate size AAAs (30-55 mm) under surveillance were re-assessed at 12 months (median duration of follow-up was 365 days). FMD deteriorated significantly during the surveillance period. Furthermore, there was a significant inverse correlation between FMD (at baseline) and AAA *growth* in the following 12 months, particularly in the subgroup of AAAs with diameter of 40-55 mm[36].

FMD as a Biomarker in the Context of PAD

There are few reports of FMD as a biomarker in the context of peripheral arterial disease. In 2007, Loffredo *et al.* reported that patients with PAD ($n=20$) had significantly lower FMD compared with matched control subjects without PAD ($n=40$)[37]. The same group subsequently utilised FMD as a surrogate marker for their investigation on the effect of propionyl-l-carnitine (an anti-oxidant) infusion in patients with PAD ($n=50$, vs 50 controls)[38]. Maldonado *et al.* examined FMD in a cohort of 50 patients with PAD (claudication $n=30$, critical limb ischaemia $n=20$) and 30 healthy individuals. They found that FMD was significantly lower in PAD patients compared to HVs, but the severity of PAD (ie claudication vs critical ischaemia) did not affect FMD[39]. Subsequently, Allan *et al.* compared the brachial artery FMD with peripheral artery tonometry (PAT) derived reactive hyperaemia index (RHI) in 26 PAD patients with claudication and 25 HVs. Patients with PAD had a significantly lower FMD than healthy subjects, but there was no difference in the RHIs between the two groups. Further, there was no correlation between the FMD and RHI in either group. They concluded that PAT is not a sensitive measure of endothelial function for PAD patients, and FMD should be the choice of measurement in this context[40].

Subsequent to these, Heinen *et al.* examined a cohort of 40 subjects in four distinct groups: symptomatic superficial femoral artery (SFA) PAD ($n=10$), symptomatic below knee (BK) PAD ($n=10$), age matched controls without PAD ($n=10$) and healthy volunteers ($n=10$). Both brachial and SFA FMD was

Table 1. Studies which examined FMD in the context of abdominal aortic aneurysm in humans

Author	Year	Context	Measure	N	Main findings
Knipp[30]	2002	*AAA #PAD ×HV	FMD NMD	*11 #9 +10	FMD and NMD are both lower in patients with AAA, and although FMD in patients with PAD is not distinguishable from HVs, NMD is significantly decreased, possibly pointing to systemic processes involved in endothelial dysfunction.
Gokce[31]	2002	*AAA #PAD	FMD NMD	*24 #100	Preoperative measurements of FMD were taken in a cohort of patients undergoing vascular interventions. Patients who suffered perioperative complications had worse preoperative FMD compared to those who didn't. No difference observed in NMD between the two groups.
Akamatsu[32]	2007	*AAA #PAD	FMD NMD	*30 #27	In patients with AAA or PAD, there was a correlation between NMD and FMD. HVs had a significantly higher FMD than patients (although the age of HVs was also significantly lower). There was no significant difference in NMD between subjects and HVs.
Akamatsu[33]	2010	*AAA/TAA #PAD	FMD NMD	*47 #26	During the follow up period (47 ± 13 months), 18 cardiovascular events were recorded. FMD and NMD were significantly lower in subjects with events. In Cox regression, lower FMD or NMD independently predicted future cardiovascular events.
Medina[34]	2010	AAA	FMD	30	AAA diameter is inversely correlated with FMD. FMD was also inversely correlated with plasma CRP levels, while AAA diameter was positively correlated with plasma CRP levels.
Sung[35]	2013	*AAA +HV	FMD	*63 +15	FMD differed between patients with large, small, and no AAAs. FMD was inversely correlated with AAA diameter. Fewer circulating endothelial progenitor cells (EPCs) were present in AAA patients than in controls. On multivariate analysis, CFUs and circulating EPCs (CD34+/KDR+) were independently, inversely correlated to AAA diameter. EPCs function (proliferation, adhesion, migration, tube formation etc) were significantly impaired in AAA patients.
Lee[36]	2017	AAA	FMD NMD FMC	162	FMD is inversely correlated with the size of AAAs. FMD deteriorates during the natural progression of AAA in an individual. FMD is inversely correlated with the future 12-month growth of AAAs. Surgical repair of AAA (open or endovascular) leads to significant improvements in FMD at 8-12 weeks. No association between NMD and FMC with AAA size or growth.

recorded in all groups at baseline, and then again in the SFA PAD group following balloon angioplasty. In addition, intimal media thickness at the site of FMD measurement was also recorded. In patients with SFA or BK PAD, both brachial artery and SFA FMDs were significantly worse than those of the HVs'. FMD correlated with intima/media or plaque thickness at the site of FMD measurement. Progressive deterioration of FMD was further observed across the segment of SFA disease (pre-, intra-, post- stenosis)[41].

A recent report by Iwamoto *et al.* examined FMD in 100 subjects (30 patients with PAD, 30 patients with Buerger's disease, age and sex matched subjects without PAD ($n=30$) or without Buerger's disease ($n=20$). FMD was worse in patients with PAD compared to the non-PAD subjects. Interestingly, they did not observe differences in FMD between patients with Buerger's disease and control subjects[42].

Surgical Intervention and the Effect on FMD

In the broader cardiovascular literature, it is known that the derangement in FMD is a reversible process[3]. Medications commonly used for cardiovascular risk prevention, such as angiotensin converting enzyme inhibitors and statins, can improve endothelial function[43-46]. However, very few studies have examined the reversibility of FMD in the context of vascular surgery intervention. These are summarised below.

In 2008, Husmann *et al.* reported a randomised controlled study of 33 patients of chronic PAD due for femoral-popliteal intervention. Patients were ran-

Table 2. Studies which examined FMD in the context of peripheral arterial disease in humans

Author	Year	Context	Measure	N	Main findings
Loffredo[37]	2007	*PAD +No-PAD	FMD	*20 +40	Patient with PAD had lower FMD compared to matched control subjects without PAD
Husmann[47]	2008	PAD	FMD	33	Patients who underwent femoral-popliteal revascularisation demonstrated significantly improved FMD
Maldonado[39]	2009	*PAD +HV	FMD	*50 +30	FMD is lower in patients demonstrating either claudication or critical limb ischaemia when comparted to HVs No difference in FMD between claudicants or critical limb ischaemia
Unal[48]	2011	PAD	FMD	54	Patients presenting with lower limb PAD due to femero-popliteal obstruction demonstrated significant improvements in FMD following femoro-popliteal bypass grafting
Jacomella[49]	2012	PAD	FMD	24	Hypertensive patients presenting with renal artery stenosis produced significantly improved FMD responses following percutaneous transluminal renal artery angioplasty
Allan[40]	2013	*PAD +HV	FMD PAT	*26 +25	When compared to healthy volunteers; PAD patients demonstrated significant reductions in FMD but not in peripheral artery tonometry (PAT) measurements of reactive hyperaemia
Heinen[41]	2015	*PAD #No PAD +HV	FMD	*20 #10 +10	In patients with SFA or BK PAD, both brachial artery and SFA FMDs were significantly worse than those of the HVs'. FMD correlated with intima/media or plaque thickness at the site of FMD measurement. Progressive deterioration of FMD was further observed across the segment of SFA disease (pre-, intra-, poststenosis). Significant improvement of SFA FMD within 24 hours of SFA balloon angioplasty to the level observed in pre-stenotic SFA segment
Iwamoto[42]	2016	*PAD #No PAD +Buerger's ^No Buerger's	FMD	*30 #30 +20 ^20	FMD was worse in patients with PAD compared to the non-PAD subjects. No differences in FMD between patients with Buerger's disease and control (no Buerger's) subjects

domised to receive either endovascular revascularisation plus best medical therapy (EV + BMT, $n = 17$) or best medical therapy only (BMT, $n = 16$). Baseline FMD did not different between the two groups. FMD significantly improved in the EV + BMT group at 4 weeks after procedure, but remained unchanged in the BMT only group[47]. Subsequently, Unal *et al.* observed significant improvement of brachial FMD in 54 patients four weeks after femoro-popliteal bypass grafting for SFA PAD[48]. In another study, Jacomella *et al.* examined the FMDs in 24 hypertensive patients with renal artery stenosis before and one day after renal artery stenosis (RAS) angioplasty. In this cohort, FMD significantly improved after RAS angioplasty, while NMD remained unchanged[49]. In the aforementioned study by Heinen *et al.*, they also observe significant improvement of SFA FMD within 24 hours of SFA balloon angioplasty to the level observed in the pre-stenotic SFA segment[41]. The OxAAA study was the first to examine the effect of AAA surgery on brachial artery FMD. FMD was measured in 50 patients before and 8-12 weeks after AAA surgery (endovascular repair, $n = 28$; open surgical repair, $n = 22$). FMD was significantly improved by AAA surgery, irrespective of the type of surgery performed[50].

These studies are summarised in **Table 1** and **2**.

Flow Mediated Constriction as a Biomarker of Disease

There are few published reports regarding FMC in cardiovascular research. After Gori *et al.* first described the method of FMC measurement in radial arteries of patients with cardiovascular risk factors, this technique was adopted by Weissgerber *et al.* who further described FMC measurement in the brachial arteries[51]. Spiro *et al.* were the first to examine brachial FMC in the context of coronary artery disease (CAD)[52]. In patients with symptomatic CAD ($n = 86$), FMC was greater in those with unstable cor-

onary disease compared with those with stable angina[53]. Norioka *et al.* reported the largest series on FMC to date. A total of 188 participants (140 smokers and 48 non-smokers) were studied. A significant correlation between body mass index and FMC was observed in male participants only[54]. In the OxAAA study, 96 patients with AAAs underwent FMC measurement at the same time as the FMD measurement. In contrast to the findings on FMD (summarised above), FMC did not correlated with AAA size or growth, and remains unchanged during AAA surveillance and after surgery[50].

Conclusion

This review highlights the literature on FMD as a biomarker in the context of abdominal aortic aneurysms and peripheral arterial disease. Although limited in numbers, these studies provide important evidence to implicate endothelial dysfunction as an underpinning mechanism of the pathologies that confront vascular surgeons. FMD should be considered as a worthy biomarker to be assessed in future research by vascular surgeons.

List of Abbreviations

FMD	Flow Mediated Dilatation
FMC	Flow Mediated Constriction
NMD	Nitro-glycerine Mediated Dilatation
NO	Nitric Oxide
eNOS	endothelial Nitric Oxide Synthase
GMP	Guanosine Mono Phosphate
PAD	Peripheral Arterial Disease
L-FMC	Low – Flow Mediated Constriction
AAA	Abdominal Aortic Aneurysm
CRP	C-Reactive Protein
EPC	Endothelial Progenitor Cells
SFA	Superficial Femoral Artery
BMI	Body Mass Index

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