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State-of-the-Art Papers

Diabetes Mellitus-Associated Vascular Impairment

Novel Circulating Biomarkers and Therapeutic Approaches

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It is widely accepted that diabetes mellitus (DM) impairs endothelial nitric oxide synthase activity as well as enhances the production of reactive oxygen species, thus resulting in diminished nitric oxide bioavailability and the consequent pro-atherogenetic alterations. Important biomarkers of the vasculature are related to endothelial dysfunction, to inflammatory and coagulation processes, and to oxidative stress in DM. Several therapeutic strategies might exert favorable effects on the vasculature of diabetic patients, such as insulin analogues, antihypertensive agents, statins, and hypoglycemic agents, whereas in spite of the prominent role of oxidative stress in diabetes, antioxidant therapy remains controversial. The use of specific biomarkers related to vascular function could be a useful therapeutic approach in such patients. (J Am Coll Cardiol 2013;62:667–76) © 2013 by the American College of Cardiology Foundation

It is well known that diabetes mellitus (DM) is a metabolic disorder characterized by increased mortality rates and importantly implicated in the atherogenetic process (1). Hyperglycemia, insulin resistance, hyperinsulinemia, hyperlipidemia, and hyperhomocysteinemia represent important pathophysiological components of DM that result in endothelial/vascular dysfunction through several underlying processes (2).

It is widely accepted that DM impairs endothelial nitric oxide synthase (eNOS) activity as well as enhances the production of reactive oxygen species (ROS), thus resulting in diminished nitric oxide (NO) bioavailability and the consequent pro-atherogenetic alterations (3). Moreover, diabetic subjects exhibit pathologically enhanced biomarkers of endothelial function, such as vascular cell adhesion molecule (VCAM)-1 and von Willebrand factor (vWF), and markers of systemic inflammation including C-reactive protein (CRP) and tumor necrosis factor (TNF)-α (4). Additionally, intrinsic properties of the injured endothelium result in vasoconstriction, smooth cell proliferation, coagulation disorders, leukocyte aggregation, thrombosis, and vascular inflammation predisposing to atherosclerosis (3).

In the present article, we review the pathophysiological role of DM in vascular dysfunction, focusing on the major novel biomarkers and briefly reviewing the current effective therapeutic approaches.

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Impaired Endothelial Function in DM

It is well established that endothelium is not just a single layer but rather a regulator exerting significant autocrine, paracrine, and endocrine actions and affecting several cell types. In addition, endothelium regulates the vascular tone via several vasoactive mediators and primarily NO. However, these functions are altered in states of DM (Fig. 1, Table 1). Hyperglycemia, insulin resistance, and elevated free fatty acids (FFAs) trigger systemic inflammation and impair NO bioavailability (5), leading to impaired endothelial function.

Insulin is a normal regulator of eNOS activation and NO production through successive phosphorylation. More specifically, insulin stimulates phosphatidylinositol 3–kinase/Akt pathway, which enhances eNOS activation and subsequent NO production. Insulin resistance in DM attenuates this pathophysiological process and suppresses the normal NO secretion (6). Furthermore, within the pathophysiological alterations of DM, FFAs are typically elevated. Consequently, NO bioavailability is further impaired, whereas oxidized low-density lipoprotein (oxLDL) formation is enhanced. Recent data support the implication of tetrahydrobiopterin (BH4) and asymmetric dimethylarginine (ADMA) in diabetic atherosclerosis (7).

Equally important, endothelial progenitor cells (EPCs) are likely implicated in diabetic vasculopathy. Endothelial progenitor cells are stem cells enrolled to repair any damage to endothelium. Clinical and experimental studies conclude that DM impairs quantity and quality of EPCs; thus there is a blunted response to vascular injury, and in this sense they propose cell-based therapy as a novel approach to patients with DM.

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Abbreviations and Acronyms

ADMA = asymmetric dimethylarginine
BH4 = tetrahydrobiopterin
CAD = coronary artery disease
CRP = C-reactive protein
DM = diabetes mellitus
eNOS = endothelial nitric oxide synthase
EMP = endothelial microparticle
EPC = endothelial progenitor cell
FFAs = free-fatty acids
FMD = flow-mediated dilation
hsCRP = high-sensitivity C-reactive protein
ICAM = intercellular adhesion molecule
IL = interleukin
LPS = lipopolysaccharide
MET = metformin
miR = micro-ribonucleic acid
MP = microparticle
NO = nitric oxide
oxLDL = oxidized low-density lipoprotein
PAI = plasminogen activator inhibitor
ROS = reactive oxygen species
TNF = tumor necrosis factor
T2D = type 2 diabetes mellitus
VCAM = vascular cell adhesion molecule
vWF = von Willebrand factor

The aforementioned pathophysiological alterations of DM damage endothelial integrity through inflammatory and oxidative processes (Fig. 2). Systemic inflammation results in migration of leukocytes into the vessel wall and in increased secretion of cytokines. Elevated levels of circulating cytokines cause—among other changes—enhanced oxLDL effected by scavenger macrophages and results in foam cell formation, which is critical in the atherosclerotic process, especially in DM.

Circulating Biomarkers Associated With Vascular Dysfunction in DM

Inflammatory biomarkers. Changes in the expression of adhesion molecules, pro-inflammatory molecules, and alterations in their regulation exist in states of DM (Table 1). The activated endothelium expresses adhesion molecules and other factors that participate in the inflammatory process and, consequently, in the pathophysiology of atherosclerosis in DM. Vascular endothelium is not only affected but also contributes through these processes. Thus, endothelial cells can be stimulated by pro-inflammatory molecules such as TNF-α and CRP to promote an atherogenic phenotype (8). The main inflammatory markers that are used and have been proved to provide prognostic information on the outcome and progression of the disease in diabetic patients are CRP, TNF-α, interleukin (IL)-6 intercellular adhesion molecule (ICAM)-1, and VCAM-1.

CRP. C-reactive protein is the most commonly used inflammatory biomarker. In addition to its role as an important inflammatory marker, it can exert modulatory effects through its presence in atherosclerotic plaques (9). C-reactive protein is an acute-phase protein, and its expression is mainly regulated by IL-6 during the acute phase with both a causative role of DM and a role associated with the risk of DM complications. More specifically, increased CRP levels are independent predictors of type 2 diabetes mellitus (T2D) in apparently healthy women, supporting the hypothesis that subclinical inflammation is an underlying factor in the pathogenesis of T2D (10). Beyond DM development, CRP predicts the outcomes in diabetic patients with cardiovascular disease. Decreased CRP levels might serve as a major predictor of successful percutaneous transluminal angioplasty outcome in diabetic patients (11), whereas high-sensitivity C-reactive protein (hsCRP) has been proven to be useful in predicting adverse cardiac outcomes (12). Given that inflammation plays a crucial role in restenosis, Paiva et al. (13) demonstrated also that diabetic patients exhibit higher pre-procedural levels of CRP and revealed a further exacerbated inflammatory response after intervention. According to data derived from the Munich Myocardial Infarction Registry (14) in diabetic patients, both a CRP level >7 mg/l and a glomerular filtration rate <60 ml/min were independent risk factors for mortality (14). Although several studies have debated the prognostic role of CRP, the relation of CRP and DM might be more complex, as previously described (15). Thus, the prognostic role of CRP after MI in diabetic patients seems to be reduced, suggesting that the burden of risk factors associated with DM might blunt the prognostic role of CRP. Recently, novel factors including genetics have made this association more complex (16).

PRO-INFLAMMATORY CYTOKINES. Other inflammatory molecules, such as pro-inflammatory and anti-inflammatory cytokines, have been evaluated or are still under investigation. Important cytokines participating in atherogenesis are IL-6 and TNF-α, with a role that is mainly associated with the risk of DM complications. Activation of inflammatory processes in DM—mainly on the basis of the increased levels of CRP, fibrinogen, IL- 6, IL-1, and TNF-α—might lead in alterations of vaso-regulatory responses, leukocyte adhesion to endothelium, and facilitation of pro-coagulant activity.

A case-control study, within the prospective population-based EPIC (European Prospective Investigation into Cancer and Nutrition)-Potsdam study (17), has demonstrated that the pattern of circulating inflammatory cytokines modifies the risk for T2D. A combined elevation of IL-1beta and IL-6 was independently associated with increased risk of T2D, suggesting that subclinical inflammatory process has a role in the pathogenesis of T2D. In a cross-sectional study of youth with and without type 1 DM, inflammatory markers were evaluated as potential contributors to accelerated atherosclerosis. Patients exhibited higher IL-6 and fibrinogen levels, independent of adiposity and glycemic control, whereas hsCRP levels were significantly higher in patients of the top 3 quartiles of glycated hemoglobin and among normal-weight subjects (18). Furthermore, after percutaneous coronary intervention, IL-6 was significantly increased in diabetic persons with peri-interventional hyperglycemic state and inversely correlated with responsiveness to clopidogrel and aspirin (19). Moreover, DM patients showed higher platelet reactivity after a 600-mg clopidogrel loading dose (compared with non-DM), and the observed increase in platelet reactivity was mainly due to a higher platelet aggregation in individuals with poor metabolic control.

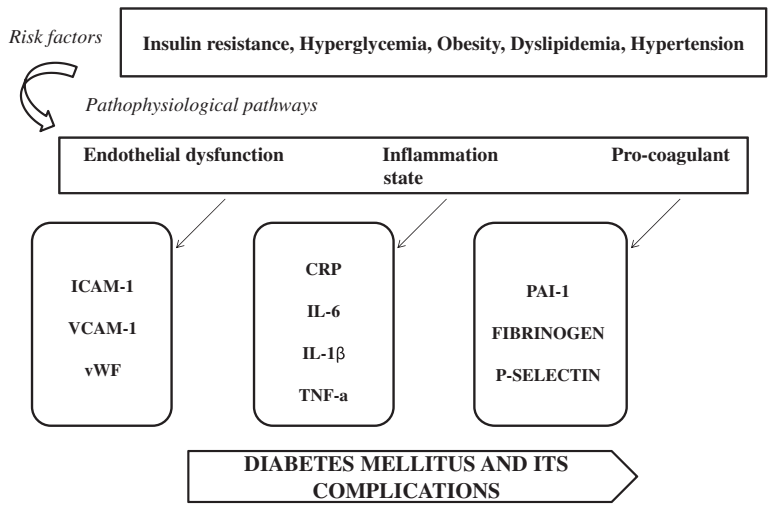

Figure 1 Diabetes-Related Factors Leading to Its Complications

Insulin resistance, hyperglycemia, obesity, dyslipidemia, and hypertension are states that accompany diabetes mellitus. Through several complex pathophysiological pathways, such factors are capable of impairing endothelial function, increase inflammatory process, and alter the pro-coagulant state. CRP = C-reactive protein; ICAM = intercellular adhesion molecule; IL = interleukin; PAI = plasminogen activator inhibitor; TNF = tumor necrosis factor; VCAM = vascular cell adhesion molecule; vWF = von Willebrand factor.

Diabetes mellitus was also a strong predictor of post-treatment platelet reactivity (multivariable analysis), whereas after analysis of interindividual time dependency of platelet reactivity, the platelet inhibition by antiplatelet treatment was mitigated in DM patients compared with non-DM. Of note, in the field of pro-inflammatory cytokines, among end-stage renal disease patients due to DM, elevated IL-6 levels have strong predictive value for poor outcome (20). With regard to the treatment of DM, it has been demonstrated to alter inflammatory cytokines. This is nicely exemplified by insulin treatment in T2D patients, which has been shown to affect the expression of IL-6 and subsequently to modify the thrombotic mechanisms in patients with coronary atherosclerosis, independent of the duration of diabetes and extent of coronary artery disease (CAD) (21).

Table 1 Mechanisms and Markers of DM-Related Endothelial Dysfunction

Mechanisms	Biomarkers	Status	Manifestation
	ICAM-1	↑	
	VCAM-1	↑	
	IL-1-IL-6	↑	
Insulin resistance	TNF-α	↑	
Abnormal cluster of hyperglycemia	MCP-1	↑	
Increased oxidative stress/ reduced nitric oxide production	Selectins (E-selectin)	↑	
	Growth factors	↑	Decreased vasodilation
Increased inflammatory status	(VEGF)	↑	Increased vasoconstriction
Elevated free fatty acids	ET-1	↑	
Glycosylated-end products	PAI-1	↑	Increased atherogenesis
Vascular smooth muscle cell dysfunction	Fibrinogen	↑	Impaired arterial remodeling
Endothelial dysfunction	vWF	↑	
	Glucose levels	↑	Increased arterial stiffness
	Insulin resistance	↑	
	HbA1c	↑	
	EPCs	↓	
	ADMA	↑	
	Homocysteine	↑	

ADMA = asymmetrical dimethylarginine; EPC = endothelial progenitor cell; ET = enthothiline; HbA1c = glycosylated hemoglobin; ICAM = intercellular cell adhesion molecule; IL = interleukin; MCP = monocyte chemoattractant protein; PAI = plasminogen activator inhibitor; TNF = tumor necrosis factor; VCAM = vascular cell adhesion molecule; VEGF = vascular endothelial growth factors; vWF = von Willebrand factor; ↑ = increased, up-regulated; ↓ = decreased, down-regulated.

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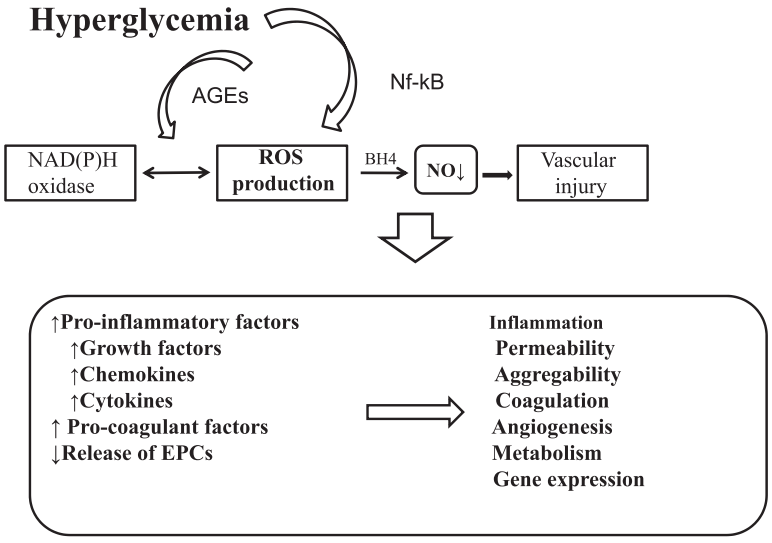

Figure 2 **Critical Role of Hyperglycemia in Atherogenesis**

Hyperglycemia plays a central role in states of diabetes mellitus. Pathways triggered by advanced glycated end products and mediated by the nuclear factor (Nf)-kB lead to increased oxidative status and thus to impaired nitric oxide (NO) production/bioavailability. As a result, numerous substances are increased, such as growth factors, cytokines, pro-coagulant factors, and others strongly related to altered underlying processes that induce and promote atherogenesis. AGE = advanced glycated end product; NAD(P)H = nicotinamide adenine dinucleotide phosphate; ROS = reactive oxygen species; EPC = endothelial progenitor cell; BH4 = tetrahydrobiopterin.

Tumor necrosis factor-α has a primary role in the regulation of immune cells; however, it represents a pleiotropic inflammatory cytokine, because it has been widely studied for its pathogenic role in disease. Increased levels of TNF-α have been found in acute and chronic inflammatory conditions in which a shift toward a pro-atherogenic lipid profile and impaired glucose tolerance occurs. It has been demonstrated that admission hyperglycemia is related to increased serum concentrations of IL-6 reduced ex vivo production of TNF-α and is associated with increased intensive care unit mortality rate in a medical intensive care unit (22). Furthermore, TNF-α levels were elevated in patients with T2D, according to a cross-sectional study. Also, TNF-α concentrations and brachial artery diameter were negatively correlated with flow-mediated dilation (FMD) and remained significantly associated with FMD after adjustment for group, age, and body mass index (23).

ADHESION MOLECULES. In the presence of risk factors such as DM, the endothelium can be activated and expresses VCAM-1 and ICAM-1, important mediators for the adhesion of leukocytes to the endothelial surface and significantly related to the risk of DM complications. In a large prospective, nested case-control study within the Nurses' Health Study (24), including women with DM, baseline median levels of the biomarkers of endothelial dysfunction were significantly higher among cases than among control subjects. Moreover, elevated E-selectin and ICAM-1 levels predicted incident of diabetes in logistic regression models after adjustment for several risk factors. However, in patients with and without diabetes presenting with unstable angina and non–Q-wave myocardial infarction there was no significant difference in levels of soluble ICAM-1 and VCAM-1 between diabetic and non-diabetic patients (25).

Coagulation-related biomarkers. Diabetes mellitus is associated with alterations in the balance of pro-thrombotic and anti-fibrinolytic state, and several molecules such as plasminogen activator inhibitor (PAI)-1, tissue factor, and vWF could reasonably serve as markers of endothelial function. Both endothelial cells and macrophages contribute to the generation of altered coagulation processes via increased expression of PAI-1, tissue factor, platelet activation, and acute phase reactions that increase levels of coagulation factors such as fibrinogen. It is worth mentioning that pro-thrombotic molecules such as PAI-1, vWF, and fibrinogen are produced under cytokine stimulation and therefore can be considered as products of the acute phase response, whereas current knowledge indicates that they are associated with the risk of DM complications. It is very important to highlight that acute phase reactions are a generalized inflammatory response to stimuli and not specific products. In the Insulin Resistance Atherosclerosis Study (26) subjects with diabetes at follow-up had higher baseline levels of fibrinogen as well as of CRP and PAI-1 than control subjects. Notably, PAI-1 levels have predicted T2D, independent of insulin resistance. Additionally, Yngen et al. (27) indicated that patients with type 1 DM and microangiopathy had remarkably elevated CRP and

E-selectin levels, whereas vWF levels did not vary between the contrasting populations. In patients with microangiopathy, thrombin-induced platelet P-selectin expression was enhanced and soluble P-selectin and soluble CD40 ligand concentrations were increased compared with the control subjects, whereas all 3 parameters were similar in patients without microangiopathy and in the control subjects. Notably, in a 9-year observational study of T2D, it has been demonstrated that vWF, E-selectin, CRP, tissue plasminogen activator, and fibrinogen are independent predictors of progression of microalbuminuria and mortality (28).

Oxidative stress biomarkers. Increased oxidative stress (associated with the complications of DM) in the vasculature is a major contributor of endothelial dysfunction in DM via the superoxide production and impairment of NO bioavailability in the vascular wall. Hohenstein et al. (29) examined eNOS expression in patients with diabetic nephropathy and noticed a strong correlation between eNOS activity and degree of proteinuria, which is indicative of glomerular endothelium function, thereby supporting the importance of increased oxidative stress as a mechanism of endothelial dysfunction in DM. Also, it has been demonstrated that circulating markers of oxidative stress, including F2 isoprostanes and antibodies against oxLDL, are increased in humans with diabetes, obesity, and insulin resistance. According to cross-sectional data derived from the community-based Framingham Offspring Study (30), across 8-epi-prostaglandin F2alpha/creatinine tertiles, the prevalence of insulin resistance increased. Thus, systemic oxidative stress seems to be related to insulin resistance in subjects at average or increased risk of diabetes.

Other circulating biomarkers related to DM. Tetrahydrobiopterin is an essential co-factor in the regulation of eNOS. Reduced synthesis or oxidative inactivation of the BH4 could also lead to reduced NO availability; thus when BH4 is limiting, NOS generates oxygen rather than NO from oxygen and nicotinamide adenine dinucleotide phosphate and is potentially related to the risk of DM complications. Tetrahydrobiopterin plasma levels along with levels in the vascular wall could reflect vascular endothelial health, given that its levels are vulnerable to acute increases in plasma glucose and might be affected early in T2D.

Moreover, clinical studies have examined the controversial role of homocysteine in diabetic atherosclerosis and complications. In a relevant study, hyperhomocysteinemia was associated with reduced creatinine clearance, potentially through endothelial dysfunction. Thus, plasma homocysteine might be an intermediate factor in the relationship between endothelial dysfunction and renal function (31). The suspected relation between hyperhomocysteinemia and ADMA should also have been noticed. Higher concentrations of ADMA have been associated with endothelial dysfunction in several cardiovascular diseases, such as hypercholesterolemia and CAD (32). This is nicely exemplified by Fard et al. (33), who demonstrated that plasma ADMA increased after a high-fat meal, and this was associated with a decrease in brachial arterial vasodilation after reactive hyperemia. Despite the promising studies conducted, recent evidence arising from the use of folates suggests that the role hyperhomocysteinemia as a risk factor for cardiovascular disease and DM should be toned down, although its measurement might be helpful for identifying subjects at greater risk of disease, who might thus benefit from a more aggressive treatment of other modifiable risk factors (34). Additionally, hyperhomocysteinemia should not be underestimated, because it might maintain interest as a marker of systemic or endothelial oxidant stress (35).

Endothelial progenitor cells are circulating cells mostly known as mediators of endothelial repair. Several of the abnormalities associated with insulin resistance, including reduced NO bioavailability and production of ROS, potentially interfere with EPC function while exhibiting a potential role in the risk of DM complications. Thus, T2D subjects seem to have reduced levels of circulating EPCs correlated with disease severity, whereas hyperglycemia might partially explain this association (36).

Recently highlighted biomarkers: micro-ribonucleic acids and endothelial microparticles. Although several biomarkers already established for their association with DM have been used to describe endothelial dysfunction in states of DM, during the last years, 2 new biomarkers have emerged as potential challengers of the classical biomarkers (Table 3).

Micro-ribonucleic acids (miRs), a class of approximately 22 nucleotide noncoding ribonucleic acids, are nowadays considered as significant modulators of several processes (37,38), because they are able to regulate gene expression. The contribution of miRs to atherogenesis is not fully evaluated; however, recent research suggests that miRs dysregulation can lead to endothelial dysfunction. The miRs exhibit either favorable or suppressive effect on eNOS activity. Actually, the overexpression of miR21 enhances NO production but mitigates endothelial cell apoptosis (39,40). By contrast, miR-221 and miR-222 overexpression lead to impaired eNOS activity and consequently decreased NO bioavailability (40). Down-regulation of numerous miRs (miR-20b, miR-21, and others) has been found in states of DM, whereas miR-28-3p was found to be enhanced (41). A combination (according to the authors) of 5 miRs (miR-15a, miR-126, miR-320, miR-223, miR-28-3p) can be adequate for a nonredundant classification. Furthermore, dysregulation of these miRs enabled the identification of 52% of normoglycemic subjects in developing DM in a 10-year period. Also, in patients with newly diagnosed DM, miR-9, miR-29a, miR-30d, miR-34a, miR-146, miR-124, and miR-375 were significantly higher compared with subjects with normal glucose tolerance (42). Although promising, the studies on miRs have shown a role only in DM complications, whereas a causative role is still not confirmed. Not only miRs but also several genetic polymorphisms have been investigated in terms of DM. Thus, polymorphisms of molecules such as osteoprotegerin (43) or that of the transcription factor 7-like 2 locus (44) could be used in the

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assessment of DM complications and the risk of DM in specific populations, respectively. Other studies have shown that genetic polymorphisms of eNOS are associated with the risk of diabetic neuropathy (45) and hypertension (46), whereas other eNOS polymorphisms might have a protective role against diabetic retinopathy (47). Recently, it was shown that a single nucleotide polymorphism on CRP gene (A3872G) might affect the duration of and partly the risk for DM (16). In addition, this polymorphism had a significant impact on CRP levels in patients with T2D exhibiting CAD.

Closely related to the miRs are the microparticles (MPs). The latter are shed membrane particles of <1 μm in diameter thought to be budded into the circulation from endothelial cells (EMPs) and various blood cells, including platelets, leukocytes, and erythrocytes (1,2,48,49). Circulating MPs constitute a heterogeneous population of different cellular origins, numbers, size, and antigenic composition. Proposed mechanisms of MP generation include apoptosis, mechanical injury, and cellular activation by cytokines (48,49). Microparticles are found in blood circulation of healthy subjects, and their number is increased in cardiovascular disease and conditions predisposing to cardiovascular disease (48,49). The number of MPs, long considered as functionally inert cell debris, is suggested as a marker of endothelial damage and platelet activation (48,49). More recently, it was appreciated that MPs harbor a number of membrane and cytoplasmic proteins and therefore could play a role as a disseminated storage pool of bioactive effectors in intercellular communication mediating effects in cardiovascular physiology and pathophysiology (48–51). Moreover, EMPs are also implicated in the trafficking of messenger ribonucleic acid and proteins between cells. Interestingly, EMPs contain nuclear acid material such as deoxyribonucleic acid, ribonucleic acid, and miR, which might be transferred to target cells (52). In the era of DM, the available studies provide promising results for the role of MPs, but currently limited to the risk of DM complications. Thus, Tsimerman et al. (53) investigated the role of MPs in DM vascular complications. The authors characterized the cell origin and pro-coagulant profiles of MPs obtained from healthy and DM subjects with CAD and DM-related complications. They demonstrated that MP characteristics are associated with the type of vascular complication and might serve as a biomarker for the pro-coagulant state and vascular pathology in patients with DM. In addition to these finding it was shown (54) that EMP (CD31+/CD42b-, CD31+/AV+) levels were higher in patients with macroangiopathy than in patients with microangiopathy and no complications. Endothelial MP level was also related to macroangiopathy in DM patients. Moreover, plasma EMPs have been associated with presence of hypertension and arterial stiffness in patients with DM (55), whereas another study has suggested that EMPs could be used as a surrogate marker of unstable plaques and might help to improve cardiovascular prediction in DM patients at intermediate risk (on the basis of the association between plasma EMP-CD144+ and coronary noncalcified plaques) (56).

Current Effective Therapeutic Approaches Targeting the "Diabetic" Vascular Function

Several therapeutic strategies might exert favorable effects (Table 2) on the vasculature of diabetic patients, such as insulin analogues, antihypertensive agents, statins, and hypoglycemic agents, whereas in spite of the prominent role of oxidative stress in diabetes, antioxidant therapy had no benefit in large randomized trials.

Hypoglycemic agents. Ersoy et al. (57) examined, among others, PAI-1 and vascular endothelial growth factor levels in obese diabetic patients who received metformin. After a 12-week treatment the aforementioned biomarkers were significantly decreased. Additionally, in patients with T2D who received metformin for 16 weeks, VCAM-1, ICAM-1, PAI-1, soluble E-selectin, and vWF were significantly downregulated, whereas markers of inflammation remained unaffected (58). After 6 months in long-acting insulin analogues either insulin glargine or detemir significantly improved biomarkers of endothelial function (ICAM-1, VCAM-1, E-selectin) as well as levels of EPCs (59), whereas high doses of insulin for 12 months significantly reduced fibrinogen, vWF, PAI-1, and thrombomodulin (60). At this point, it is very important to discuss the association of insulin and inflammation, because it is much-debated, giving attention to the recent hypothesis for the potential anti-inflammatory role of insulin. Thus, Dandona et al. (61) investigated whether insulin reduces the magnitude of oxidative, nitrosative, and inflammatory stress and tissue damage responses induced by endotoxin (lipopolysaccharide [LPS]). For this purpose, the authors recruited healthy subjects who received intravenous injection with LPS, along with others who received infusion with insulin in addition to the LPS injection. The concomitant infusion of insulin resulted in a significant reduction, considering the already known effects of LPS injection, in ROS generation and the total prevention of the increase in thiobarbituric acid–reacting substances, NO metabolites, and FFAs concentrations. These effects were related to a significant reduction in the magnitude of increase in macrophage migration inhibition factor, myoglobin, and visfatin levels (independent of glucose levels). By contrast, insulin was unable to prevent or reduce the magnitude of increase of specific pro-inflammatory cytokines, raising further questions about the effect of more prolonged infusions and higher doses of insulin.

Statins. A prospective double-blind trial investigating the effect of atorvastatin in endothelial function of diabetic persons has suggested that markers of endothelial function as well as markers of inflammation were significantly improved after a 12-week treatment of atorvastatin (62). Additionally, 90 days of simvastatin resulted in amelioration of TNF-α, CRP, and several types of ILs (IL-6, IL-2, IL-1b), which

Table 2 Agents Interfering With Endothelial Dysfunction in Diabetes Mellitus

First Author (Ref. #)	Population	Treatment	Effect on Endothelial Function and/or Inflammation	p Value
De Jager et al. (58)	390 patients with T2D (30–80 yrs)	Metformin vs. placebo	vWF, VCAMI-1, E-selectin, and PAI-1 reduced but CRP, ICAM-1 remain unchanged	Within reference values
Jankovec et al. (60)	13 obese patients with T2D	Long-term insulin pump	Thrombomodulin, PAI-1, vWF reduced	<0.01
Economides et al. (62)	Patients with T2D vs. subjects at risk for T2D	Atorvastatin	FMD improved in both groups, CRP and TNF-α decreased at the at-risk group	<0.05
Tehrani et al. (65)	20 patients with T1D and dyslipidemia	Placebo vs. atorvastatin	p-selectin, tissue factor decreased, PAI-1, hsCRP, fibrinogen unchanged in atorvastatin group	Within reference values
Neri et al. (66)	46 patients with T2D, 46 patients with IGT, and 46 healthy individuals	Antioxidant supplementation (Vit C, Vit E, *N*-acetylcysteine)	VCAM-1, vWF, ET-1, and oxidants reduced, NO increased	<0.05
Schram et al. (70)	70 hypertensive with T2D	Hydrochlorothiazide vs. candesartan vs. lisinopril	VCAM-1 and ICAM-1 reduced, CRP and vWF unchanged in all groups	Within reference values
Beisswenger et al. (72)	45 patients with T2D	Prandial + basal insulin vs. basal insulin	TNF-α, IL-6, hsCRP more attenuated in prandial + basal insulin group	<0.01, <0.01, and <0.001, respectively
Tousoulis et al. (73)	35 patients with newly diagnosed T2D	MET vs. MET + atorvastatin	TNF-α reduced in MET + atorvastatin group	<0.05
Bellia et al. (74)	29 patients with T2D with mild untreated dyslipidemia	Rosuvastatin vs. simvastatin	FMD improved in the simvastatin group, no changes in CRP	<0.01
Aversa et al. (75)	20 patients with T2D without erectile dysfunction	Sildenafil	FMD increased, ET-1, CRP, IL-6, VCAM-1, ICAM-1 reduced	0.01
Bank et al. (76)	34 patients with T2D and hypertension	Carvedilol vs. metoprolol	FMD improved more in carvedilol group	<0.001
Martina et al. (77)	24 male patients with T2D and hypertension	L-arginine + *N*-acetylcysteine vs. placebo	CRP, ICAM-1, VCAM-1, PAI-1 reduced	<0.05, <0.05, <0.01, and <0.05, respectively
Chakraborty et al. (78)	208 patients with T2D	Metformin vs. placebo	CRP decreased	<0.05
Karagiannis et al. (79)	1,170 subjects with T2D	PIO monotherapy vs. PIO combined with other oral anti-diabetic drugs	hsCRP reduced	<0.01
Pop-Busui et al. (80)	27 subjects with T2D	Rosiglitazone vs. glyburide	hsCRP and vWFag reduced and adiponectin increased in rosiglitazone group	<0.03 and <0.05, respectively
Haffner et al. (81)	Patients with T2D	Rosiglitazone vs. placebo	CRP reduced	<0.01

ACE = angiotensin converting enzyme; CRP = C-reactive protein; FMD = flow-mediated dilation; hsCRP = high-sensitivity C-reactive protein; IGT = impaired glucose tolerance; MET = metformin; NO = nitric oxide; PIO = pioglitazone; T1D = type 1 diabetes mellitus; T2D = type 2 diabetes mellitus; Vit = vitamin; other abbreviations as in Table 1.

supports the aforementioned scenario, with regard to the effect of statins on endothelial function (63). By contrast, several conflicting studies have found no important improvement of either endothelial function or systemic inflammation in diabetic persons treated with statins (64,65).

Antioxidants. A single-blind study recently enrolled patients with T2D and examined the variance of certain circulating markers after 15 days of antioxidants. Actually, plasma levels of oxidants as well as biomarkers of endothelial dysfunction (vWF, VCAM-1, enthothiline-1, NO) were significantly decreased (66). In addition, Haidara et al. (67), in a diabetic rat model using alpha-tocopherol and vitamin C, have shown that administration of antioxidants significantly decreased vWF, plasma soluble thrombomodulin, and fibrinogen. However, the protective effect of antioxidants on vascular endothelium is still unidentified.

Renin-angiotensin system-related agents. Wago et al. (68) examined the endothelial function and systemic inflammation of subjects under the influence of telmisartan. The FMD significantly increased after a 12-month treatment with telmisartan, whereas adiponectin and hsCRP levels decreased; Flammer et al. (69) confirmed these findings. After 4-week treatment with losartan, FMD significantly increased and isoprostane as a marker of oxidative stress reduced, whereas CRP levels remained unaffected. In addition, a double-blind study (70) observed that

Table 3 Important Biomarkers in Diabetes Mellitus

Biomarker	Diabetes-Related Characteristics	Most Reliable Methods
CRP (hsCRP)	Increases rapidly, long rising periods, stability in plasma Attenuates NO production Decreases endothelial NO synthase Triggers oxidation of low-density lipoprotein cholesterol Induces PAI-1expression Stimulates the release of matrix metalloproteinase-1 Activates macrophages to secrete tissue factor Upregulates the expression of adhesion molecules in endothelial cells	Ultrasensitive solid-phase ELISAs Immunoturbidimetric CRP assays Immunonephelometry (laser nephelometry)
TNF-α	Growth stimulating properties and growth inhibitory processes self regulatory properties Cytokines triggering Inflammation and apoptosis Expressed in cells such as: B-cells, T-cells, macrophages, monocytes, mast cells, neutrophils and adipocytes	Enzyme-linked immunosorbent Assays (ELISA)
IL-6	Affects extracellular matrix dynamics at mesangial and podocyte levels Stimulates mesangial cell proliferation Increases fibronectin expression Enhances endothelial permeability Induces the production of adrenocorticotropin Affects insulin sensitivity probably mediated by adenosine monophosphate-activated protein kinase Affects glucose homeostasis and metabolism directly and indirectly by action on skeletal muscle cells, adipocytes, hepatocytes, pancreatic cells, and neuroendocrine cells.	ELISAs
ICAM-1	Promotes the recruitment of mononuclear cells in diabetic glomeruli Role in glomerular hyper-filtration Interactions of lymphocyte function-associated antigen-1 Binds with the site 117–133 of the fibrinogen gamma chain Leads to changes through the protein kinase C pathway, cAMP, phospholipase A2, Ca^{+2} and proteosomes (extracellular signal transportation)	ELISAs Flow cytometry
VCAM-1	Interacts with secreted protein acidic and rich in cysteine Interacts with VLA-4	ELISAs Flow cytometry
Fibrinogen	Lower platelet inhibition Interacts with ICAM-1	Automated clotting rate assays Immunoassays (ELISA or nephelometric) Automated immunoassays of total fibrinogen The Clauss fibrinogen assay (on the basis of the thrombin clotting time)
Endothelial microparticles	Thrombosis, cell inflammation, angiogenesis and cell-to-cell communication Apoptosis Factor XI-dependent pro-coagulant properties Impaired no release	Flow cytometry
miRs	Affect pancreatic b cells and insulin-target tissues Interaction with TGF-beta Affect insulin secretion	Real-time qPCR Microarrays RNA sequencing Northern blot In situ hybridization

Characteristics and measuring methods.

CA^{+2} = calcium doubly charged positive ion; cAMP = cyclic adenosine monophosphate; ELISA = enzyme-linked immunosorbent assay; miR = micro-ribonucleic acid; PAI = plasminogen activator inhibitor; qPCR = quantitative polymerase chain reaction; RNA = ribonucleic acid; TGF = transforming growth factor; VLA = very late antigen; other abbreviations as in Tables 1 and 2.

aggressive antihypertensive therapy with either candesartan or lisinopril reduced VCAM-1 and ICAM-1, yet CRP and vWF remain unchanged. We recently found that perindopril significantly improved endothelial function in diabetic patients (71).

Conclusions

The association between DM and vascular dysfunction is under continuous investigation, and several circulating biomarkers have been proposed as indicators of endothelial dysfunction. Important biomarkers of the vasculature are related to endothelial function, inflammatory and coagulation processes, and oxidative stress. These biomarkers could explain the different pathophysiological aspects in patients with DM and, along with the newly investigated biomarkers, could provide useful guidelines for the treatment of these patients.

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