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IM - REVIEW

Biomarkers of vascular disease in diabetes: the adipose-immune system cross talk

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Abstract
Experimental and clinical studies aimed at investigating the mechanism(s) underlying vascular complications of diabetes indicate that a great number of molecules are involved in the pathogenesis of these complications. Most of these molecules are inflammatory mediators or markers generated by immune or adipose tissue. Some of them, i.e. resistin and sortilin, have been shown to be involved in the cross talk between adipocytes and inflammatory cells. This interaction is an attractive area of research, particularly in type 2 diabetes and obesity. Other proteins, such as adiponectin and visfatin, appear to be more promising as possible vascular markers. In addition, some molecules involved in calcium/phosphorus metabolism, such as klotho and FGF23, have an involvement in the pathogenesis of diabetic vasculopathy, which appears to be dependent on the degree of vascular impairment. Inflammatory markers are a promising tool for treatment decisions while measuring plasma levels of adipokines, sortilin, Klotho and FGF23 in adequately sized longitudinal studies is expected to allow a more precise characterization of diabetic vascular disease and the optimal use of personalized treatment strategies.

Keywords Diabetes · Vascular complications · Biomarkers · Atherosclerosis

Introduction

The increasing prevalence of type 2 diabetes mellitus (T2DM) is associated with an overall rise of vascular diseases [1], such as coronary artery disease (CAD), peripheral artery disease (PAD) and stroke. Taken together, these conditions are the leading causes of morbidity and mortality in western countries. In addition, they induce a major impact on public health, in term of quality of life and economic burden. Assessing the risk of diabetic complications may facilitate the use of preventive strategies and innovative treatments.

To this end, over the last two decades, plasma levels of several molecules were investigated as potentially associated with the pathogenesis and the evolution of vascular damage, particularly in diabetes mellitus. In clinical practice glycated hemoglobin, albuminuria, serum creatinine and estimated glomerular filtration rate (eGFR) are now routinely measured in diabetic patients. Despite their utility in the prediction of cardiovascular outcomes [2–6], these molecules remain important, being the result of the vascular damage rather than being involved in its pathogenesis [4, 7]. Some data seem to underline the role of T2DM as a promoter of inflammation with an apparently causal link between high levels of inflammatory markers and the occurrence of vascular complications. The adipose tissue is emerging as a new source of inflammatory molecules with an attitude to self-potentiating effects due to a cross talk between adipocytes and inflammatory cells. In addition, the inflammation worsens the insulin resistance while hyperglycemia seems to increase the incidence of major vascular events per se [8].

In this scenario, a growing number of plasma biomarkers are being tested as prognostic and, in some case, therapeutic tools for diabetic vascular complications.

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The aim of this review is retracing the role of molecules identified in the last years, to facilitate an overview of the main results and encourage further investigations in this field.

Potential diagnostic and prognostic biomarkers of vascular diabetes complications

Inflammation-related biomarkers

C-reactive protein (CRP) is widely used as a reliable marker of inflammation. This protein has a key role in the modulation of innate immunity since it promotes the activation of phagocytes and of the complement pathway and the production of cytokines [9]. These effects may account for CRP involvement in atherosclerosis and diabetes mellitus [10]. High sensitivity CRP (hs-CRP), increased plasma levels are considered an independent cardiovascular risk factor which can increase the accuracy of Framingham score in risk prediction [11]. The pro-inflammatory effect of CRP has been attributed to its binding to lectin-like oxidized LDL receptor-1 (LOX-1), expressed by endothelial cells. In fact, this process promotes the expression of more LOX-1 scavenger receptors, with a positive feedback loop. Moreover, it directly determines an augmented intake of proatherogenic and proinflammatory molecules inside endothelial cells, such as oxidized LDL (OxLDL) or electronegative L5 LDL whose plasma levels are particularly increased in patients with high cardiovascular risk, in smokers and in patients affected by dyslipidemia and diabetes mellitus [12]. Furthermore, high levels of OxLDL and L5 LDL increase the CRP levels in atheromatic plaque thus sustaining the vicious cycle between inflammation and atherosclerosis [13]. CRP facilitates the development of foam cells and cause platelet adhesion [10]. Further studies have also demonstrated an enhanced CRP-mediated uptake of OxLDL uptake by macrophages through FCyRs that leads to an overexpression of NF-kB [13]. Furthermore, high hs-CPR is markedly related to endothelial dysfunction in diabetic patients [14]. The very low specificity of CRP limits its use, since hepatic and local production of CRP constitutes general response to any kind of inflammatory trigger [15].

Patients affected by diabetic PAD often undergo endovascular revascularization procedures. This approach is frequently affected by a higher rate of post-procedural complications such as major adverse limb events (MALE) and major adverse cardiovascular events (MACE). Elevated plasmatic levels of CRP show a linear association with worse vascular outcomes in diabetic PAD [10] being an independent predictor for limb amputation in diabetic patients [16].

Pentraxin 3

Pentraxin 3 (PTX3) is an acute phase reactant of the same family of CRP. It seems to act as a local inflammation marker produced by endothelial, smooth muscle and dendritic cells, macrophages, neutrophils when they are activated by tumor necrosis factor (TNF)-α, interleukin (IL)-1β and other toll-like receptors agonists [15]. PTX3 has been detected at high concentrations in human carotid plaques and coronary atherosclerotic lesions of patients with acute myocardial infarction and unstable angina [17].

A causal link between PTX3 and endothelial damage has been hypothesized in vascular complications of diabetic patients [18].

Tumor necrosis factor-α

There is evidence suggesting that TNF-α may play an important role in the pathogenesis of atherothrombosis of diabetic patients [10]. Higher levels of TNF-α are associated with higher failure rates of lower extremity endovascular revascularization and increased risk of MACE and MALE [10]. Among diabetic patients, those who are affected by diabetic ulcer foot present a higher concentration of TNF-α [19]. However, the causative link between high TNF values and adverse events still needs to be demonstrated. Indeed, anti-TNF strategies have been thought to be beneficial in several inflammatory diseases including atherosclerosis [20]. However, the negative effect on lipid profile and the lack of positive outcome detected in patients with heart failure [20] limited further studies on the therapeutic approach targeting TNF- α [11].

Potential benefits have already been shown with other anti-inflammatory drugs.

Colchicine, a classic old drug used for chronic inflammatory pathologies, is correlated, in these diseases, with better cardiovascular outcomes [21]. Similar benefits were also documented in patients at risk who have been treated with low doses of methotrexate [11].

Interleukins

Interleukin-1 (IL-1) family is constituted by IL-1 α and IL-1β, which have a pro-inflammatory effect, and by the IL-1 receptor antagonist, IL1-Ra that counteracts the effect of IL-1.

IL-1 pro-inflammatory properties result from the induction of leukocyte adhesion molecules, from the expression of prostaglandin-E2 and from cellular release of histamine, which promotes vasodilatation and permeability [22].

In diabetic patients, IL-1 contributes to pancreatic cell apoptosis leading to hyperglycemia, which results in an inflammatory burden on endothelium [22]. Moreover, the

therapeutic reduction of IL-1 concentrations (through the administration of IL-1Ra) improves glycemic control, reduces CRP and decreases the incidence of cardiovascular disease [23].

IL-1α promotes inflammation, through the induction of IL-6 with an indirect pro-inflammatory effect that is also shared by the isoform IL-1β [11].

The role of IL-1β in diabetic atherosclerosis and its modulation as possible therapeutic strategy is receiving increasing attention. In fact, IL-1β is implicated in the development of the inflammasome of atherosclerotic plaques [11]. Moreover, IL-1β production enhances by "autoinduction" both the expression of its own gene and of several other genes in cells involved in atherosclerosis [24]. This molecule decreases cardiac contractility by worsening cell injury associated with reperfusion and remodeling [25]. This cytokine stimulates smooth muscle cell proliferation [26] and regulates leukocyte adhesion molecules as ICAM-1 and VCAM-1; furthermore, it promotes the induction of cyclooxygenase-2 (COX-2) nitric oxide synthase (iNOS). Finally, IL-1β activates cells belonging to the innate immunity system [11]. The role of IL-1β in the development and progression of atherosclerosis led to test a specific treatment to antagonize its effects [11]. Canakinumab is a human monoclonal antibody which selectively inhibits IL-1β and has a positive effect in IL-1β mediated pathologies such as juvenile inflammatory arthritis [27], and acute gouty arthritis [11]. Specifically, in cardiovascular diseases this monoclonal antibody allows a reduction in plasma concentrations of hs-CRP, IL-6 and fibrinogen, with possible benefits for patients who present a residual inflammatory risk, defined as an hs-CRP greater than 2 mg/L, after the optimization of standard therapy [11].

Furthermore, IL-1 inhibition treatment decreases the risks of overall MACE, unstable angina, and breakthrough or recurrence of heart failure [28].

IL-18 is an additional member of the IL-1 superfamily. It works as a starter of the inflammatory process through the induction of interferon gamma (IFNγ) and the over-activation of Nf-kB [22].

Interleukin 18 is activated together with IL-1β [11]. Higher levels of IL-18 correlate with increased incidence of both diabetes mellitus and diabetic complications [22].

In addition, the role of chemotaxis is being investigated in atherosclerosis. In particular, the monocyte chemoattractant protein-1 (MCP-1/CCL2), a chemoattractant of monocyte, and its receptor C–C chemokine receptor type 2 (CCR2) seem to have an important role in atherosclerosis [29]. Recent studies have focused on monocyte behavior in cardiovascular disease [11].

In diabetes mellitus, there are increased plasma concentrations of MCP-1, which promote monocyte migration into the vascular wall and foam cell generation. Higher MCP-1 levels are common in patients with high levels of hs-CRP and in those with carotid artery thickness [30].

In addition, epicardial adipose stores of patients with critical CAD overexpress MCP-1 mRNA that could partially account for the increased incidence of myocardial infarction and stroke [31] observed in these patients.

The binding of MCP-1 to CCR2 receptor plays a role in the development of insulin resistance, obesity and diabetic major vessel complications [32].

This recognition of the role of MCP-1 in the inflammatory and atherosclerotic process led to development of new possible therapeutic strategies, such as monoclonal antibodies aimed to inhibit CCR2 and reduce inflammation [11]. Additionally, targeting MCP-1 could become a potential new direct treatment of diabetes mellitus and its major complications [33].

Moreover, since the overexpression of this chemoattractant protein might be involved in the pathophysiological process of diabetes mellitus, it becomes an available marker to guarantee a prompt diagnosis preventing the development of vascular complications [32].

IL-6 is a pro-inflammatory cytokine produced by lymphocytes and macrophages, which is able to stimulate the immunity system. High levels of this interleukin are predictor of MACE and MALE in diabetic patients with PAD as well as of poor outcome of endovascular revascularization procedures [10].

IL-6 is the main starter for inflammasome development and the importance of this cytokine in atherosclerosis is clearly documented by the lower incidence of cardiovascular adverse events occurring in patients with a particular loss-of-function variant of IL-6 receptor that inhibits the proinflammatory downstream pathway [34].

Tocilizumab, a monoclonal antibody that inhibits IL-6, has been shown to have a potential benefit in several diseases and to reduce CRP increase after primary coronary artery procedures [11]. However, blocking both IL-6 signaling pathways (the classic and the trans-signaling one), results in an impairment of immunity as well as of insulin sensitivity [24].

New approaches to selectively inhibit part of the IL-6 pathways are being investigated. The inhibitor of p38 MAP kinase (losmapimod) does not seem to affect the IL-6 pathway in a manner resulting in a reduction of MACE [11].

High mobility group box-1

The high mobility group box-1 (HMGB-1) is a nuclear protein that regulates gene expression and active pro-inflammatory responses in damaged and necrotic endothelial cells [35]. It is an inflammatory mediator able to induce the secretion of other inflammatory molecules such

as IL-6 and TNF-α by neutrophils and macrophages [36]. There is evidence of a cross talk among HMGB-1 and pro-inflammatory cytokines, suggesting a contribution of this pathway to the inflammatory process and to atheroma formation [37]. Indeed, several studies have documented a correlation between HMGB-1 levels, diabetes and its complications [38]. In addition, Oozawa et al. found that HMGB1 plasma levels were increased in diabetic patients with PAD [39]. We demonstrated in a large population of T2DM patients that HMGB-1 plasma levels are significantly increased in PAD patients with a positive correlation with clinical severity of vascular impairment [40].

In addition, HMGB-1 increases the expression of osteoprotegerin (OPG) [41] whose role as biomarker of vascular impairment is described below.

Biomarkers generated by adipose tissues

Adipokines

Adipokines are bioactive substances produced by visceral adipose tissue. These molecules have a role in the regulation of the balance between pro-inflammatory and anti-inflammatory effects of adipose tissue (Fig. 1). There are experimental and clinical data showing that this balance is compromised in T2DM [42]. Adipose tissue dysfunction contributes to insulin resistance, vascular injury and consequent vascular disease. Several studies are investigating the possible role of adipokines as biomarkers for diabetic vascular complications. Evidences are still controversial about the negative, positive or independent association between the levels of different adipokines and cardiovascular risk factors.

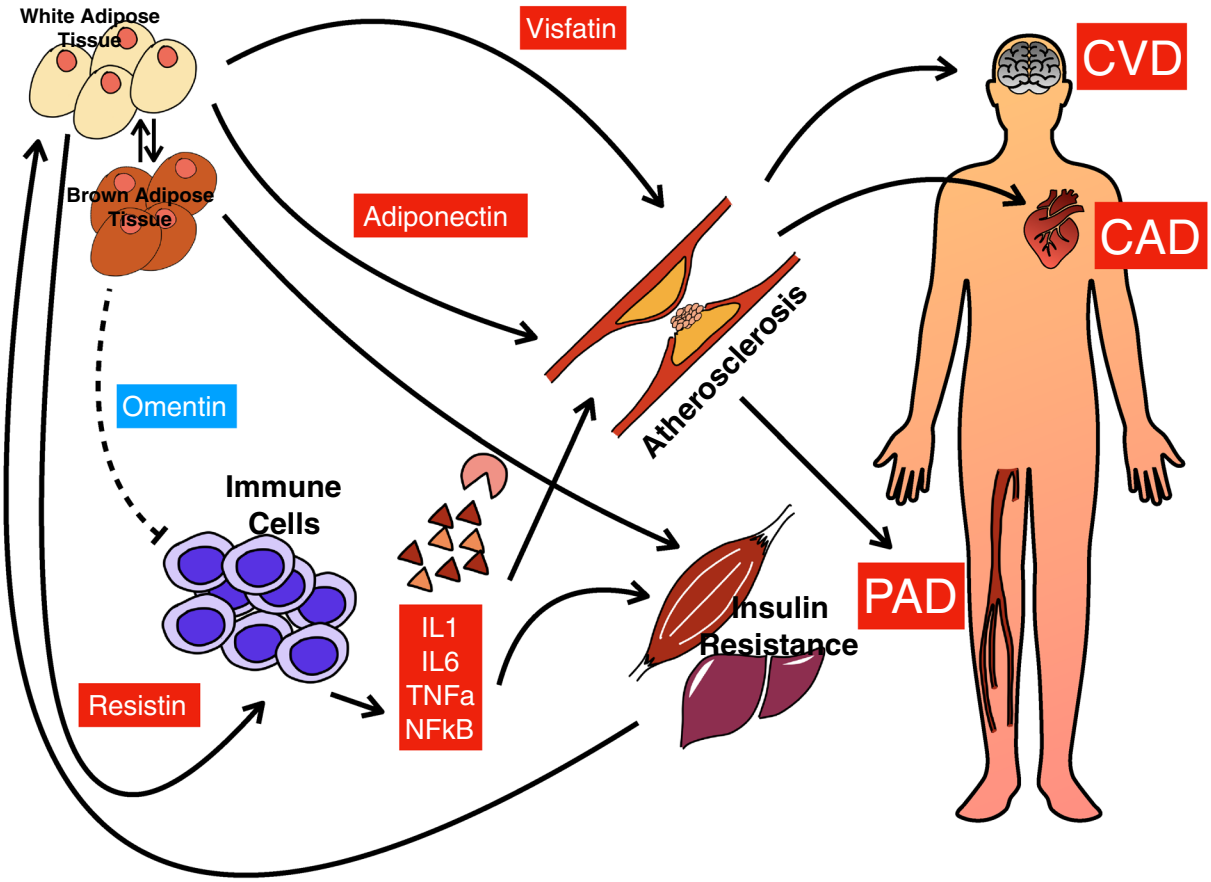

Fig. 1 Effect of different adipokines on inflammation and vascular complications. Different molecules, produced by different phenotypes of adipocytes, on inflammation, atherosclerotic plaque growth, glucose metabolism. Adipose-immune system cross talk influences the phenotype of adipose tissue and has an impact on the risk of coronary artery disease (CAD), cerebrovascular disease (CVD) and peripheral artery disease (PAD). *IL1* interleukin 1, *IL6* interleukin 6, *TNF*α Tumor Necrosis Factor-α, *NFkB* nuclear factor kappa-light-chain-enhancer of activated B cells

Adiponectin is a protein composed of 244 amino acids, encoded by a gene located on chromosome 3q27, which is related to T2DM and cardiovascular disease [43]. It is mainly synthesized in white adipose tissue. Several studies suggest that hypoadiponectinemia is associated with CAD indicating a protective role of adiponectin against the development of atherosclerosis process [44, 45]. Low serum adiponectin level may be considered an additional cardiovascular risk factor also for diabetic patients [46]. Al-Daghri et al. have studied this association at the genetic level. They found a link between a gene variant of adiponectin and CAD [47]. Other studies, performed in a population at intermediate-high CAD risk, documented that high plasma levels of adiponectin in diabetic patients are associated with higher rates of MACE rates [48]. Thus, adiponectin levels might need to be differently interpreted in populations with different degree of vascular impairment.

Omentin is a protein composed of 313 amino acids, encoded by a gene present on the chromosomal region 1q22-q23, that is related to T2DM [49]. Omentin mRNA is mostly expressed in the fraction of the vascular stroma of visceral adipose tissue, rather than in subcutaneous adipose tissue and in mature adipocytes. It has also been identified in other tissues, such as endothelium, epicardial adipose tissue, thymus, small intestine, colon, ovary, lung and placenta. There are two homologous isoforms: omentin-1, the most common form in human plasma, and omentin-2, which shares 83% of amino acids with isoform 1. Basic and clinical research has shown an anti-inflammatory effect of omentin-1 and an inverse correlation between its plasma levels and insulin resistance, diabetes, obesity and metabolic syndrome [50]. In mice, Yamawaki et al. have shown that treatment with omentin prevents inflammation of the endothelium by blocking the activation of JNK and NF-kB induced by TNFα and reducing the expression of adhesion molecules [51]. Several studies have investigated the relationship between omentin-1 and CAD, stroke, and PAD [52] in diabetic patients. These studies, mostly cross-sectional and conducted on small and heterogeneous populations, provide discordant data about the negative, positive or independent correlation between omentin-1 levels and cardiovascular risk. Menzel et al. suggest that the different metabolic conditions may variably influence the levels of omentin and explain these results [53]. Therefore, prospective data are necessary to define the role of this adipokines as biomarker of an earlier diagnosis and management of vascular disease in diabetic patients.

Resistin is a protein synthesized in white adipose tissue, which is able to induce insulin resistance in muscles and liver and to promote the formation of early atherosclerotic lesion by proinflammatory pathways [54]. Several studies have documented that resistin plasma levels are elevated in obese and diabetic patients [55]. In particular, On et al. reported that resistin levels were higher in diabetic patients with CAD than in those without CAD, and the pre-procedural resistin levels were higher in diabetic patients that manifested restenosis after stenting [56]. Indeed, resistin seems to induce the proliferation of smooth muscle cells and may have a role in restenosis of coronary lesions in patients with diabetes. Therefore, resistin assay may be useful in diabetic patients for assessing the risk of cardiovascular disease as well as the risk of restenosis after stenting [57].

Visfatin is an adipokine produced and secreted by visceral fat, whose plasma levels are correlated with obesity, insulin resistance and T2DM [58]. Several studies documented a role of visfatin as inflammatory mediator. It is involved in endothelial dysfunction and atherosclerotic plaque instability [59]. Mazaheroun et al. have found that visfatin plasma levels were high in patients with acute myocardial infarction (AMI) and found that these levels were highly sensitive and specific for this condition [60]. Visfatin seems to play a role in the pathogenesis of vascular damage in diabetic patients. Further investigations are needed to confirm these data and verify a potential diagnostic role of this adipokine.

Biomarkers related to lipid metabolism

Sortilin

Sortilin is a protein of 95 kDa encoded by the gene SORT1 located on chromosome 1. This protein is manly expressed in hepatocytes and some studies have evidenced its role in apolipoprotein trafficking inside hepatocytes. Membrane sortilin promotes the uptake of LDL by hepatocytes via an LDL-receptor (R)-independent mechanism and also by macrophages, in the process of foam cell formation in atheroma [61]. Furthermore, there is evidence of a role of sortilin in intracellular cytokine traffic and in platelet activation [62]. Based on these data several researchers have investigated an association among sortilin, atherosclerosis, diabetes and cardiovascular complications [63]. Oh and colleagues [64] reported that circulating sortilin levels are increased in diabetic patients with CAD and in patients with CAD risk factors. Our group found that circulating sortilin levels are independently associated with PAD in a statin-free diabetic population [65]. Additionally, we found that sortilin levels correlate with PAD severity in diabetic patients, suggesting a dose-dependent relationship. The significance of these interesting findings needs to be strengthened by prospective studies.

Biomarkers related to calcium/phosphorus metabolism

Osteoprotegerin

OPG is a member of the Tumor Necrosis Factor (TNF) receptor family involved in bone turnover and vessel

calcification. Several studies have documented the role of OPG in the progression of atherosclerosis in diabetic patients [66]. OPG has also been described as an independent risk factor for disease progression in patients with CAD [67]. In PAD, there are contrasting data on the role of OPG plasma levels. Recent studies demonstrated higher OPG concentrations in T2D patients with PAD than in those without PAD [40]. However, no data are available on the possible coexistence of CAD in these relatively small patient populations. Thus, whether OPG levels might have a different behavior in diabetic vs non-diabetic or in CAD vs PAD subjects still needs to be demonstrated.

Klotho

The lower serum levels of phosphorus detected in diabetic patients have suggested an unbalance of phosphorus regulation in these patients [68]. The phosphate homeostasis is guaranteed by several hormones and factors such as vitamin D, growth hormone, thyroid hormone, calcitonin, glucocorticoids, atrial natriuretic factor, parathyroid hormone (PTH) and FGF23 [69]. The precipitation of calcium salts in arterial walls leads to vascular calcification. This has been shown to be an independent risk factor of cardiovascular disease and mortality [70]. Calcium precipitation is promoted by a dysfunction of mineral metabolism and both low and high bone-turnover are associated to a higher mineral deposition in blood vessels. T2DM is correlated to a state of low bone turnover [71] and, additionally, high PTH serum levels are associated with early vascular calcification in lower limbs of T2DM patients. In these subjects, tibial artery calcification is a predictor of PAD, diabetic ulcer foot and MALE [72]. Vitamin D deficiency is a main trigger of PTH elevation, thus suggesting that vitamin D supplementation may be beneficial in reducing vascular complications of diabetic patients [72].

FGF-23/α-klotho axis is an adjunctive regulator of mineral metabolism. The perturbation of this recently described pathway seems to accelerate arterial calcification and progression of vascular complications [73]. Indeed, elevated serum concentrations of FGF23 and low levels of circulating Klotho are markers of cardiac and large vessel complications in pre-diabetes and diabetes [74].

A number of studies document a strong association between FGF23 and cardiovascular adverse events in T2DM. Elevated serum concentrations of this hormone are correlated with a high incidence of MALE and mortality in diabetic patients as well as with high incidence of diabetic foot [66].

Klotho also plays a significant role in glucose metabolism and regulation [75]. Despite a possible promotion of insulin resistance, due to a decreased glucose cell uptake [75], its interference with insulin/IGF-1 pathway causes an inhibition of AKT kinase. This prevents the phosphorylation of FOXO transcription factor and inhibits the expression of catalase or mitochondrial manganese superoxide dismutase which cooperate in the reduction of reactive oxygen species [75].

The role of Klotho in diabetes is also suggested by the fact that Klotho is a protective factor against diabetic ulcer foot, it attenuates the progression of diabetic nephropathy and is an independent protective factor against macrovascular major adverse events (stroke and myocardial infarction) [76].

Atherosclerotic inflammation reduces the expression of Klotho in arterial wall cells probably by the expression of NF-kB, specifically through cytokines of TNF-α superfamily [77]. The lack of protective role of Klotho causes an endothelial dysfunction leading to a degenerative process of vessels wall such as endothelial cells apoptosis, decreased expression of endothelial cadherin, smooth muscle cells calcification, increase of adhesion molecules as VCAM-1 and ICAM-1, oxidation, decrease of nitric oxide and loss of endothelium integrity and function [78]. To confirm the protective role of Klotho, the infusion of exogenous Klotho reverses these degenerative effects likely through the induction of nitric oxide production and release [79]. These evidences suggest a possible implication of Klotho as predictor of sub-clinical atherosclerosis, since a reduction of the hormone serum levels is a marker of coronary disease, heart failure, stroke and peripheral artery disease [80].

Exosomes

Exosomes (EXOs) constitute a subgroup of extracellular vesicles (EVs), rich in bioactive molecules such as DNA, messenger RNA, micro-RNA (miRNA) and other proteins. Several findings show their role as drivers of intracellular communications. Many researchers have investigated a possible correlation between EXOs and CVD [81–83]. Indeed, in response to stress or injury conditions, contents of EXOs may be up- or down-regulated, giving a signal and this may be used to drive the diagnosis and prognosis for CVD. However, methods of EXOs collection, isolation, and purification are still undergoing standardization [84].

Among the identified EV RNAs, miRNA has been closely associated with CVD. Different miRNAs have different roles and implications [15]. For example, miRNA-26 has likely a role in the modulation of inflammation and leukocyte adherence [85]. Micro RNA-100, according to intravascular ultrasound discoveries that correlate its levels with atherosclerotic plaque composition [86], reduces the expression of mammalian target of rapamycin (mTOR) signaling a pathway promoting more stability of the coronary plaque [15]. An elevation of miRNA-126 concentrations was observed in diabetic patients with a strict glycemic control through an optimization of anti-diabetic treatment. This molecule

downregulates vascular inflammation and reduces the tissue factor expression in vessels with an antithrombotic effect [87].

Potential therapeutic implications

The occurrence of T2DM and related complications seems to be directly associated to inflammation which worsens the hyperglycemic state and insulin resistance. Moreover, the growing fat mass in obese patients is one important source of chronic inflammation in diabetic patients. The unbalance between the fast growth of adipose tissue and the ineffective vascular support leads to tissue hypoxia that generates and sustains subclinical inflammation [8]. Other assumed causes of this low grade pro-inflammatory process, in diabetic patients, are periodontitis [88] and diet-related gut microbiota unbalance [8]. In addition, several molecules, such as CCL2 (MPC-1), CCL5 (RANTES), CXCL8 (IL-8), TNF-α, IL-6, IL-1, CRP, are implied in this pro-oxidant chronic phenomenon which increases the incidence of vascular adverse events [8].

Clinical evaluation of inflammatory status: in which patients and when

Patients with PAD and diabetic PAD can be divided in two different groups: the asymptomatic and the symptomatic patients such as those with claudication [89].

It has been shown that, in patients with asymptomatic PAD, plasma levels of inflammatory markers are similar to those we find in control cases, while patients with intermittent claudication have higher levels of CRP and IL-6, and a lower flow mediated dilatation (FMD) than asymptomatic ones [90]. These data seem to indicate that the inflammatory status and the endothelial function are related to the severity of PAD [90]. This hypothesis is supported by the fact that ankle-brachial pressure index (ABPI), which represents the degree of circulatory impairment in the affected limb is positively correlated with plasma levels of CRP, soluble intercellular adhesion molecule-1 (sICAM-1) and Soluble vascular cell adhesion molecule-1 (sVCAM-1), and positively correlated with flow-mediated dilatation (FMD) [90].

The degree of systemic inflammation also has an inter-individual variability depending on the severity of the inflammatory status that is influenced by the quantity of atherosclerotic plaques and the quality of the composition of these lesions [90]. In fact, symptomatic claudicant patients are characterized by a systemic increased activation of neutrophil, augmented concentrations of adhesion molecules (soluble I-CAM and V-CAM), thromboxane, endotelin-1, and IL-8 with an associated endothelial dysfunction [90]. In particular, these patients present a chronic sustained inflammation with transient recidivate burdens that promote the maintenance and the amplification of this pro-inflammatory substrate increasing the cardiovascular risk [90]. Moreover, these recurrent acute inflammation loads and impairment of endothelial function are markedly reduced after a procedure of revascularization. Or after an adequate aerobic rehabilitation program which decreases the ischemic insults occurring during walk [90]. Revascularization is an effective therapeutic procedure to restore a valid blood flow to ischemic tissues of lower limbs in diabetic PAD. However, the stressful stimulus on arterial wall provoked by ballooning and stent placement (particularly in long extended stenosis) promotes local inflammation with an increase of inflammatory proteins [10]. We propose to test the hypothesis that inflammatory markers may be useful to personalize the optimal timing of PAD revascularization procedures. Patients who after the optimization of pharmacological and rehabilitation treatment have persistently high hs-CPR, IL6 and TNFα should have an earlier revascularization and should be treated with stenting, whenever possible. Furthermore, they should receive a narrower follow-up than the others, given the high risk of MALE and MACE. In this view the monitorization of inflammatory markers might also help deciding in which patients the use of anti-inflammatory agents might be most helpful [91]. Also, antidiabetic agents have different anti-inflammatory properties.

Rizza et al. have proved that treatment with the thiazolidinedione, a class of insulin-sensitizing drugs, increases the expression and secretion of adiponectin [92]. Other studies have documented that pioglitazone, a thiazolidinedione, improves cardiovascular outcomes in diabetic patients [93] an effect that may be related to adiponectin levels. In the same way, fenofibrate seems to increase adiponectin levels. Studies show a reduction of cardiovascular events and an improvement of angiogenic repair in ischemic limbs in patients with T2DM treated with this drug [94].

A number of novel approaches were made recently available such as the biologic therapies targeting molecules [95], lipid mediators (phospholipase inhibitors and antileukotrienes [95] or intracellular signaling pathways (NADPH oxidase, p38 mitogen-activated protein kinase, phosphodiesterase [96], MAP kinase [95]. This scientific effort indicates the role attributed to inflammation in atherosclerosis and the search by the scientific community of ways to slow down the development and the progression of this degenerative process [96].

Conclusion

The assay of several cytokines, such as sortilin, omentin, OPG, klotho, IL-6, TNF-α and especially hs-CRP can be used to identify diabetic patients having an inflammatory

Table 1 Effect of different cytokines on diabetes vascular complications

	Predictive	Prognostic	Therapeutic
Adiponectin	↓Serum levels are associated with CAD	↑↑Serum levels are related to the severity of CAD	Pioglitazone ↑adiponectin levels and is associated improves cardiovascular outcomes in diabetic patients Fenofibrate ↑adiponectin levels and is associated a reduction of cardiovascular events and an improvement of angiogenic repair in ischemic limbs in patients with T2DM treated with this drug
Omentin	↑/↓Serum levels are associated with CAD and PAD	↑Serum levels are related to the severity of PAD	In mice, treatment with omentin prevents inflammation of the endothelium by blocking the activation of JNK and NF-kB induced by TNFα and reducing the expression of adhesion molecules
Resistin	↑↑Serum levels in CAD	↑↑Serum levels preprocedural in patients with restenosis after stenting	–
Visfatin	↑↑Serum levels in AMI	–	–
Ctrps	↑/↓Serum levels are associated with CAD and PAD	–	Some evidences in mice
Sortilin	↑Serum levels in CAD and in PAD	↑Serum levels are related to the severity of PAD	–
HMGB-1	↑Serum levels in CAD and in PAD	↑Serum levels are related to the severity of PAD	–
OPG	↑Serum levels in PAD	–	–
CRP	CRP promotes a major accuracy of risk estimation if used as complementary tool of Framingham score	↑CRP is related to worse vascular outcomes in diabetic PAD CRP is an independent predictor of amputation in diabetic patients	–
Pentraxin 3	↑Concentrations are detected in human carotid plaques and coronary atherosclerotic lesions during acute myocardial infarction and instable angina	Pentraxin 3 has a supposed role in vascular complications of diabetic patients	–
Interleukins	↑IL-18 is correlated with major incidence of diabetes mellitus and the vascular complications related to this disease ↑IL-6 correlates to a higher incidence of endovascular revascularization procedure failure	–	The administration of IL-1Ra improves glycemic control, reduces levels of CRP and decreases the incidence of cardiovascular disease Canakinumab allows a reduction in plasma concentrations of hs-CRP, IL-6 and fibrinogen, with possible benefits for patients who present a residual inflammatory risk; IL-1 inhibition treatment decreases the risks of overall MACE, unstable angina, and breakthrough or recurrence of heart failure;
FGF23	↑FGF23 and ↓circulating Klotho are markers of MALE, diabetic ulcer foot, mortality and cardiac and large vessels complications in pre-diabetes and diabetes	–	
Klotho	↓Klotho is related to coronary disease, heart failure, stroke and peripheral artery disease ↓Klotho is associated to a more severe grade of the coronary stenosis ↓Klotho is connected to a more intense severity of disease	–	Klotho is a protective factor against diabetic ulcer foot, it attenuates the progression of diabetic nephropathy, and it protects from macrovascular major adverse events (stroke and myocardial infarction) in patients affected by type 2 diabetes mellitus

Table 1 (continued)

	Predictive	Prognostic	Therapeutic
TNF-α	↑TNF-α determines more incidence of failure of lower extremity endovascular revascularization procedures and a major occurrence of MACE and MALE Among diabetic patients, those who are affected by diabetic ulcer foot present a higher concentration of TNF-α	–	Indeed, anti-TNF strategies have been thought to be beneficial in several inflammatory disease as atherosclerosis

pattern that might be directly dependent from diabetic vasculopathy and contribute to worsen its prognosis (Fig. 1; Table 1). These assays may be helpful in subjects with advanced disease to better assess their risk and possibly to guide therapeutic decisions. Their combined use with the assay of other molecules such as adipokines, klotho and FGF23 may help defining better risk prediction models and possibly personalizing the use of innovative strategies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Statement of human and animal rights The procedures employed to draft this manuscript respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2000, as well as the national law.

Informed consent For this type of article, formal consent is not required.

Consent for publication All authors have read the paper and agree that it can be published.

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