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Angiogenic biomarkers in Hereditary Hemorrhagic Telangiectasia and other vascular diseases.

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Running title: Biomarkers in vascular diseases

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HIGHLIGHTS:

- Angiogenic pathways are implied in multiple cardiovascular diseases
- The evidence of angiogenic biomarkers for managing vascular diseases is scarce
- Here, the most relevant potential angiogenic biomarkers with clinical impact in vascular diseases are discussed
- Hereditary hemorrhagic telangiectasia (HHT) is used as a model of vascular disease with angiogenic disturbances
- This information will help clinicians dealing with these patients and researchers to focus their investigations

Abstract

Biomarkers are new tools framed in precision and personalized medicine. Hereditary hemorrhagic telangiectasia (HHT) is a rare genetic vascular disease with disturbances in the angiogenic pathways. Descriptive evidence supports that some angiogenesis-related molecules are differently detected in HHT patients compared to healthy subjects. These molecules are also related to diagnosis, prognosis, complications and therapy monitoring in other common vascular diseases. Despite the need for improving knowledge before applying them in daily clinical practice, there are good candidates to be considered as potential biomarkers in HHT and other vascular diseases. In the present review, the authors aim to summarize and discuss current evidence regarding the main putative angiogenic biomarkers by describing the biological role of each biomarker, the evidence related to HHT and their potential use in this and other common vascular diseases from a clinical point-of-view.

Keywords: Hereditary hemorrhagic telangiectasia, Biomarkers, Translational medicine, Angiogenesis, Rare diseases.

Abbreviations

ACVRL1, Activin A receptor type II-like 1 gene; ALK1, Activin receptor-like kinase 1; AM, adrenomedullin; ANGPT, angiopoietin; AVM, arteriovenous malformation; BMP9, Bone morphogenetic protein 9; CXCR-4, chemokine receptor type 4; DDP4, dipeptidyl peptidase 4; EC, endothelial cell; ESS, Epistaxis Severity Score; ENG, Endoglin; *ENG*, Endoglin gene; FGF2, fibroblast growth factor 2; GDF-2, growth differentiation factor 2; HF, heart failure; HHT, Hereditary hemorrhagic telangiectasia; HR, hazard ratio; NO, nitric oxide; OR, odds ratio; PDGF, Platelet-derived growth factor; PDGFR, PDGF receptor; PI3K, phosphatidylinositol 3-Kinase; PlGF, placental growth factor; PoPH, portopulmonary hypertension; PTX3, pentraxin 3; SDF-1, stromal cell derived factor 1; TGF-β, transforming growth factor β; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; VM, vascular malformation.

1. - Introduction

Vascular diseases are the main cause of death in developed countries [1]. Despite much progress has been made to improve the management of these diseases, it is difficult to predict those patients at high risk of mortality. Biomarkers are a relatively new clinical toolset used in the current precision medicine approach. This is a medical model that proposes the customization of healthcare, with medical decisions, treatments or practices being tailored to a subgroup of patients, instead of a "one-drug-fits-all" model. In precision medicine, individual biomarkers are often employed for selecting appropriate and optimal therapies and guiding treatment decisions [2]. In fact, the National Institutes of Health (NIH) working group standardized the definition of a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [3]. Consequently, the use of biomarkers related to angiogenic disturbances could be useful for anticipating morbidity or for tailoring therapies in patients with vascular diseases.

Hereditary hemorrhagic telangiectasia (HHT) or Rendu–Osler–Weber syndrome (ORPHA 774) is a rare autosomal dominant vascular disease characterized by systemic telangiectasia and larger vascular malformations (VMs) [4]. HHT can be diagnosed using either the Curaçao clinical criteria (recurrent epistaxis, cutaneous/mucosal telangiectasia, visceral VMs, and a first-degree family member with HHT) or through molecular genetic test [5, 6]. Mutations in the endoglin (*ENG*) and activin A receptor type II-like 1 (*ACVRL1*) genes are detected in approximately 90% of cases submitted for molecular diagnosis and cause HHT1 and HHT2, respectively [7, 8]. Endoglin (ENG; encoded by *ENG*) is an auxiliary co-receptor at the endothelial cell (EC) surface that promotes BMP9 (Bone morphogenetic protein 9) signaling through the activin receptor-like kinase 1 (ALK1; encoded by *ACVRL1*). Thus, HHT is considered a disease of the hub formed by BMP9/10–ENG–ALK1–Smads, with high impact in angiogenesis [9].

Hence, HHT is a model of vascular disease with disturbances in angiogenesis supporting the usefulness of angiogenic biomarkers [2, 10, 11]. Thus, uncovering angiogenic biomarkers in vascular diseases could help to hypothesize about HHT,

and vice versa. However, the evidence of the role and clinical utility of angiogenic biomarkers in vascular diseases is scarce and scattered. Moreover, the lack of available proteomic datasets specific for HHT or other vascular diseases, probably hampers an optimal biomarkers selection. Here, the state-of-the-art of potential angiogenic biomarkers in the management of HHT and other vascular diseases is addressed, providing a new perspective on their future benefits in clinical practice.

2. - Material and methods.

We performed an unrestricted search on PubMed/MEDLINE electronic bibliographical database through August 2022. We focused on angiogenesis-related molecules considering HHT as a model of vascular disease with angiogenic disturbances. The search was performed using the MeSH terms "biomarkers" and "hereditary hemorrhagic telangiectasia" obtaining 206 articles. According to the author's expertise we selected only those studies with the following criteria: i) biomarkers measured in blood, ii) evidence in humans, iii) potential use in clinical practice. Reference lists of retrieved articles and review articles were manually searched to extend the original search. Disagreements on study data extraction were resolved by consensus or by discussion. Finally, 12 biomarkers were found, including 27 articles related to HHT. To assess the potential role of these biomarkers in other vascular diseases, 35 new specific studies were manually selected. In order to contextualize and define the selected biomarkers and the involved pathways, additional articles were considered.

3. - Vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) family

Definition and pathway: VEGF is a heparin-binding, endothelial cell-specific growth factor capable of inducing cell proliferation and chemotaxis of ECs leading to angiogenesis [12]. It is a member of the PDGF family that includes VEFG-A to VEGF-E, PDGF and placental growth factor (PlGF) [12, 13]. Most attention is focused on VEGF-A due to its key role in regulating angiogenesis during homeostasis and disease. VEGF-A binds two tyrosine kinase receptors, VEGF receptor 1 and 2 (VEGFR1, VEGFR2). VEGFR1 is expressed in a wide range of

cells while VEGFR2 is mainly expressed in ECs. The angiogenic effect of VEGF-A is predominantly mediated by VEGFR2 while VEGFR1 down-regulates pro-angiogenic effect of VEGF-A [14, 15].

PDGF is mainly secreted by platelets, although other cell types (ECs, epithelial cells and macrophages) are involved in its synthesis. PDGF binds to PDGF receptor (PDGFR), a naturally repressed tyrosine kinase receptor. The binding between PDGF and PDGFR de-represses its tyrosine kinase activity contributing to physiological functions such as cell proliferation and vessel formation [16]. Placental growth factor (PlGF) is secreted from different cellular sources and binds to VEGFR-1 replacing vascular VEGF-A, that is then more available to binds to VEGFR-2, enhancing angiogenesis. PlGF is also able to induce other angiogenic factors including VEGF, fibroblast growth factor-2 (FGF-2), PlGF-B, and stimulates macrophage proliferation, who also releases angiogenic factors [18].

Evidence in HHT: VEGF has been studied in HHT patients with conflicting results. Cirulli *et al* and Sadick *et al* found increased levels of VEGF in plasma and tissues from HHT patients compared to healthy subjects, with no differences between HHT1 and HHT2 [19-21]. However, recent studies have not found statistically significant differences between blood levels of VEGF in HHT patients compared to healthy controls or in HHT severity [22, 23]. The use of anti-VEGF monoclonal antibodies such as bevacizumab, both systemic and nasal, is being studied in HHT patients since 2012 [24]. Liu *et al* found increased levels of plasma VEGF after 8-10 months of bevacizumab intranasal injection for preventing epistaxis in HHT patients. They found a strong correlation between plasma VEGF levels and post-treatment reduction of mild bleedings frequency and a moderate correlation between plasma VEGF levels and post-treatment reduction of mild and moderate bleedings duration [25]. Despite these promising results as a biomarker, the correlation between plasma VEGF levels and disease severity or response to anti-VEGF treatment in HHT patients is not well established. However, PlGF did not show differences when used as a plasma biomarker in HHT patients compared to healthy controls [22].

Evidence in other vascular diseases: In diabetic retinopathy, serum and tear VEGF concentration is correlated with the presence and severity of the disease [26]. In preeclampsia, an endothelial-dysfunction-related disease, and other placentation disorders, some angiogenic biomarkers could aid in the diagnosis and prognosis [27]. Levine *et al* showed an increase of the soluble form of VEGFR1 (sVEGFR1) few weeks before the onset of the clinical disease and a parallel decrease of free VEGF and PlGF (attributable to the binding of the soluble factor) [28]. Besides, preeclampsia screening scores including PlGF serum levels during the first trimester in a low-risk pregnant women population, demonstrated good performance (93% sensitivity with 5% false-positive rate) [29]. In patients with essential hypertension, elevated VEGF serum levels correlate with cardiovascular risk, early microvascular injury and target organ damage. Accordingly, hypertension treatment significantly reduced VEGF levels [30].

4. – Angiopoietin-2 (ANGPT-2)

Definition and pathway: Tie2 (encoded by *TEK* gene) is a tyrosine kinase receptor located predominantly on EC that stabilizes the vasculature increasing EC survival, adhesion, and cell junction integrity. ANGPT1 is produced by perivascular cells and binds to Tie2 receptors being critical for vessel maturation, whereas ANGPT2 is a proangiogenic glycoprotein that works as an antagonist of ANGPT1 blocking the phosphorylation of Tie-2 and promoting vessel regression in the absence of VEGF [11, 31]. Both BMP9/10 and ANGPT2-Tie2 pathways are interconnected, as the first represses ANGPT2 endothelial expression [15].

Evidence in HHT: Reduced plasma levels of ANGPT2 were observed in 62 HHT patients compared with controls (n= 38), with statistically significant lower levels in HHT2 patients (n= 30) compared with HHT1 patients (n= 32) **[**32]. Steineger *et al* found a positive correlation between ANGPT2 and Epistaxis Severity Score (ESS) and a negative one with hemoglobin levels in 75 HHT patients [22]. The administration of monoclonal ANGPT2-blocking antibodies resulted in resolution and prevention of retinal arterio-venous malformations (AVMs) in this mouse model, providing a new therapeutic target [11].

Evidence in other vascular diseases: ANGPT2 is involved in endothelial physiology and is usually elevated in most cardiovascular diseases [33]. In a cohort of 65 patients with pulmonary embolism, the highest levels of ANGPT2 were associated with pulmonary embolism severity, right ventricular dysfunction and pulmonary hypertension; an ANGPT2 level > 4101pg/ml had an OR of 7.4 (1.53-12.5) for intensive care unit admission [34]. Huang *et al* also found ANGPT2 serum levels as an independent predictor of mortality in 118 acute pulmonary embolism patients (30-day and long-term mortality) [35]. Patel *et al* uncovered raised levels of ANGPT2 as predictors of myocardial infarction in a small group of 11 hypertensive patients, proposing its potential utility as a biomarker of subclinical atherosclerosis [36]. In peripheral arterial disease and chronic kidney disease, increased ANGPT2 serum levels also correlated with an increased cardiovascular risk with higher risk of major adverse cardiovascular events and all-cause mortality [37, 38]. In diabetes, serum ANGPT2 levels are found to be elevated in type 2 diabetic patients with retinopathy compared to those without this microvascular complication [33]. Pöss *et al* revealed increased serum levels of ANGPT2 in 132 patients with acute heart failure (HF) compared to healthy controls, and worse prognosis in those patients with higher levels at discharge (increased risk of death with levels above 2.500pg/ml, HR=8.8 [2.48-31.16]) [39]. Lastly, in 469 preeclampsia low-risk pregnant women, significant lower ANGPT1/ANGPT2 ratios at 25 and 28 gestation weeks were observed in the preeclampsia group [40].

5. - Bone morphogenetic protein 9 (BMP9) or growth differentiation factor 2 (GDF2)

Definition and pathway: BMP9, also known as growth differentiation factor 2 (GDF2), is a cytokine of the transforming growth factor β (TGF-β) superfamily, mainly produced by hepatocytes, and by the lungs and brain at lower levels. It binds to ALK1, which is expressed mainly in the endothelium [41]. These two proteins are BMP9 is present in the blood circulation under both homodimeric and heterodimeric forms and it is challenging to measure accurate circulating levels [9, 41-44]. Specifically, BMP9 and 10 bind to the receptor complex composed of ALK1,

endoglin and BMPRII with higher affinity than TGF-β. When this binding occurs in the EC, VEGFR1 expression increases, ANGPT2 decreases and phosphorylation of PTEN occurs, triggering vascular quiescence and maturation. Conversely, when ALK1 or ENG lose their function, such as in HHT patients, signaling through this pathway is diminished and leads to an increase in the angiogenic response [9, 41, 42, 45].

Evidence in HHT: Mutations in the BMP9 gene result in clinical manifestations of HHT and are referred to HHT type 5 (OMIM # 615506) [46]. Some patients with *GDF2* (GDF2 gene) homozygous mutation and both pulmonary arterial hypertension and HHT-phenotype have been reported [47]. Wetzel-Strong *et al* couldn't find differences in BMP9 plasma levels neither between HHT and controls, nor among HHT patients with different visceral involvement [23].

Evidence in other vascular diseases: Circulating BMP9 levels have been studied in patients with pulmonary hypertension, observing markedly lower levels in portopulmonary hypertension (PoPH) patients compared to healthy controls, and compared to patients with liver disease without pulmonary hypertension [48]. Marked lower BMP9 levels were found in fasting patients with anthropometric measures of metabolic syndrome, type 2 diabetes or essential hypertension [49-51].

6. - Soluble endoglin (sENG):

Definition and pathway: ENG is a homodimeric transmembrane glycoprotein that acts as an auxiliary receptor for cytokines of the TGF-β family. It is expressed mainly in vascular endothelium and plays a key role in vascular physiology. There is a circulating form containing the extracellular domain named soluble ENG (sENG), shed from membrane-bound ENG by the proteolytic activity of the matrix metalloprotease [52]. sENG induces context-dependent effects depending on the presence of endogenous transmembrane ENG. It has been postulated that the molecular mechanism of action is related to its capacity to antagonize the function of membrane-bound ENG acting as a scavenger, trapping circulating ENG ligands (mainly BMP9) and secondarily tapering the TGF-β intracellular signaling [44].

Evidence in HHT: Plasma levels of sENG in both HHT1 (n = 32) and HHT2 (n = 30) resulted lower than in healthy donors (n = 38) and were significantly lower in HHT1 patients than in HHT2 [32]. *ENG* haploinsufficiency as the underlying cause of HHT1 strongly supports these results [33]. Patients with liver VMs have higher levels of sENG, but since these VMs are more common in HHT2 than in HHT1 patients, these differences are probably a confounding factor due to the genetic basis of HHT, rather than a further increase above normal levels [23].

Evidence in other vascular diseases: increased sENG serum levels have been related to the development of preeclampsia and PlGF-to-sENG ratios measured between 20 and 25 weeks of gestation showed a good predictive performance with a likelihood ratio of 57.6 [53]. In cardiovascular disease, elevated sENG levels have been observed in hypercholesterolemia patients, associated to atherosclerotic cardiovascular risk, and correlated with carotid intima-media thickness as a marker of subclinical atherosclerosis [54]. Elevated serum sENG levels are present in patients with diabetes mellitus, especially in those at early stages of diabetic retinopathy [55]. Finally, this biomarker is increased and significantly correlated (r=0.689) with left ventricle end-diastolic pressures as a marker of cardiac failure, and sENG levels decrease in response to diuretic treatment [56].

7. – Adrenomedullin (AM)

Definition and pathway: AM is a peptide hormone that belongs to the amylin/calcitonin gene-related peptide family, mostly secreted by vascular endothelial and smooth muscle cells [57]. This hormone has multiple actions that are exerted through combinations of the calcitonin receptor-like receptor (CLR), and either receptor activity-modifying protein 2 (RAMP2) or RAMP3, also known as AM1 and AM2 receptors, respectively [58]. AM plays critical roles in blood vessels, with vasodilatory properties that help regulating vascular stability and permeability through modulation of the endothelial barrier and circulatory homeostasis management [59, 60]. AM also has an integral role in linking blood flow with nitric oxide (NO) production and vasodilatation [60]. Besides vasodilatation, AM plays an

important role in preservation of endothelial integrity, and it is known that increased plasma AM levels correlate with excessive fluid volume [61, 62]. In addition, AM directly stimulates angiogenesis and its inhibition reduces angiogenesis in animal models and EC [59, 63].

Evidence in HHT: Given the vasodilating properties of AM, its enhanced local expression in endothelial and smooth muscle cells may cause variaions in blood flow and contribute to VMs development in different scenarios [64, 65]. Higher AM serum levels have been found in 45 HHT patients compared to 50 healthy volunteers. Moreover, this higher expression has been confirmed in telangiectasias biopsies from eight HHT compared to skin from five control patients [10]. These results are consistent with previous studies where mouse and human EC with compromised BMP9 signaling, led to higher AM levels [9, 10, 66]. Thus, elevated levels of AM found in HHT patients may result from known dysregulation of the signaling hub formed by BMP9–Endoglin–ALK1–Smad [9, 47]. Moreover, other research has shown that AM-induced angiogenesis in EC is mediated by activation of phosphatidylinositol 3-Kinase (PI3K), which is overstimulated in HHT [67-69]. Although how AM exactly interacts with EC biology in patients with HHT has not been elucidated, all these data support that AM could be used as a novel biomarker.

Evidence in other vascular diseases: Levels of AM are markedly increased in patients with HF, so AM has been proposed as a biomarker of fluid overload and correlates with aggravation of signs and symptoms of remaining congestion, and in consequence, with disease severity [70]. Moreover, increased levels of AM have been related with greater risk of readmission due to decompensated HF in 1230 patients with acute HF, resulting a useful biomarker to identify patients at risk of rehospitalization because suboptimal decongestion [71]. In diabetes, AM levels are increased in type 1 diabetic patients with microangiopathy and in all type 2 patients. Additionally, it has recently emerged as a potential biomarker for the diagnosis of pancreatic cancer-induced diabetes [72].

8. - Pentraxin 3 (PTX3)

Definition and pathway: The pentraxin family includes molecules involved in innate immunity and acute-phase response widely known as C reactive protein (CRP) [73]. PTX3 is a CRP-related protein classified in the long pentraxin group and implicated in inflammation, tissue repair and extracellular matrix organization, innate immunity and cancer. It rises in response to different inflammatory and/or infectious diseases [22, 73]. It is produced at the site of the lesion by different cell types, including inflamed EC, and has the capacity to act as a soluble pattern recognition molecule (PRM) for selected microorganisms, producing activation of the complement and binding to cellular receptors of the monocyte/macrophage system [73, 74].

Evidence in HHT: PTX3 resulted significantly higher in 75 HHT patients compared to 16 healthy controls and showed significant correlation to epistaxis severity grade, needs of blood transfusion and hemoglobin levels [22]. Thus, PTX3 can be a potential biomarker of epistaxis severity in HHT patients.

Evidence in other vascular diseases: PTX3 has shown to be a useful biomarker in many vascular diseases, partly reflecting vascular inflammation [22]. For instance, early measurement in patients with myocardial infarction and in large cohorts of healthy patients, high PTX3 levels predict short and long-term cardiovascular and all-cause mortality [73]. In patients with chronic HF (both reduced and preserved ejection fraction), high PTX3 levels were a better predictor of adverse outcomes (including all-cause mortality, cardiovascular mortality and hospitalization due to HF) than brain natriuretic peptide [75, 76]. In the GenPE study, a positive correlation between PTX3 levels and preeclampsia (OR=1.03, 1.03-1.10) or HELLP syndrome (OR=1.13, 1.08-1.18) was found [77].

9. – Fibroblast growth factor 2 (FGF-2)

Definition and pathway: FGF2, also named basic FGF (FGFb), belongs to the FGF family proteins. FGF2 is ubiquitously expressed, but despite acting in both autocrine and paracrine manners, the mechanism through which it is secreted remains unknown. Once FGF2 is secreted, it interacts with FGF receptor 1 (FGFR1) to

trigger its dimerization and phosphorylation. Consequently, it activates different effectors, such as the proliferation cascade RAS/RAF/MEK/MAPK [78]. FGF2 has been characterized as a potent inductor of cell proliferation and differentiation; furthermore, it delays senescence, inhibits apoptosis and participates in tissue repair and homeostasis. FGF2 was the first factor described as an angiogenic inductor, promoting angiogenesis not only by itself but boosting VEGF effect. Since FGF2 has high affinity to heparin, there is an important reserve associated to the extracellular matrix that is released after VEGF-induced proteolysis, amplifying the VEGF effect [78-79].

Evidence in HHT: Choi *et al* performed an *in vitro* assay in which *ALK1*-null ECs increased migration in response to FGF2 compared with *ALK1*-het ECs [80]. This means that ALK1 inhibits FGF2-induced angiogenesis, relating FGF2 to HHT. Since FGF2 is secreted during inflammation, it might impair BMP9 signaling in ECs and trigger vascular lesions after a second hit event in HHT patients [80, 81]. Further investigation is needed for assessing both serum and urine levels of FGF2 and its combination with other factors as potential biomarkers for HHT.

Evidence in other vascular diseases: El-Raggal *et al* revealed that FGF2 (and VEGF) serum levels were increased in children with infantile hemangioma (n= 48) or VM (n= 12) compared to 40 healthy matched controls. In fact, the combination of both FGF2 and VEGF serum levels was useful to differentiate VMs from infantile hemangiomas with a good sensitivity (85.42%) and specificity (100%) [82]. Furthermore, Yang *et al* investigated the role of serum and urine FGF2 in infantile hemangiomas (n= 97), VMs (n= 25) and controls (n= 48). Serum and urine FGF2 levels were significantly higher among children with hemangioma and VM than controls [83].

10. - Transforming growth factor β (TGF-β) and Activin A

Definition and pathway: TGF-β and other members of its family, such as activins, are multi-functional cytokines that regulate cell growth and differentiation, apoptosis, extracellular matrix production, cellular immune responses and

angiogenesis [84]. TGF-β is known to be a powerful angiogenic factor and a mediator of vascular remodeling. There are 3 isoforms of TGF-β (β1, β2 and β3), being present in different tissues and having distinctive functions. TGF-β1 is considered the most implicated in angiogenesis as knock-out mice models showed impaired hematopoiesis and vascular development [84, 85].
The interaction with activin receptors mediates endothelial proliferation and angiogenesis but also cell differentiation, repair, and apoptosis [86].

Evidence in HHT: Several groups have evaluated TGF-β1 serum levels in patients with HHT compared to controls, showing controversial results: Sadick *et al* reported higher levels of TGF-β1 in 31 HHT patients compared to controls while Letarte *et al* found lower plasma levels of this biomarker in HHT1 patients (n=34) compared to controls, while HHT2 group (n= 23) was unchanged [2, 20, 87]. Wetzel-Strong *et al* (n= 42 HHT patients) and Steineger *et al* (n= 75 HHT patients) observed no significantly differences [22, 23]. These last authors also correlated the TGF-β1 levels with the need for blood transfusions and the ESS [22]. Wetzel-Strong *et al* studied related biomarkers as TGF-β2 and the soluble form of the receptor of TGF-β type 3 (TGF-β3R) without identifying differences between HHT patients and healthy controls [23].
The only study related to HHT patients (n= 75) assessing activin A, did not show differences in serum levels compared to healthy controls; however, activin A levels were significantly correlated with the ESS and hemoglobin levels [22].

Evidence in other vascular diseases: There are conflicting results of the relationship between TGF-β and preeclampsia with reports of high, normal and low levels in this population [88]. On the other hand, activin A levels are repeatedly found raised in patients who developed preeclampsia during pregnancy and are also higher compared to gestational hypertensives and normotensive controls [88]. In patients with idiopathic and heritable pulmonary arterial hypertension, higher TGF-β1 levels were found compared to healthy controls, and positively correlate with functional class and mortality (TGF-β1 levels >3.74 ng/ml have a 2-year mortality of 25% compared to 8% in patients below this cut-off) [89]. TGF-β is also an

atherosclerosis marker correlated with advanced disease, but low circulating levels of this biomarker are associated with unstable plaques because it also promotes a stable lesion phenotype [90]. Activin A has been proposed as a useful biomarker related to worse prognosis in diastolic dysfunction HF or acute kidney failure (the latest, tested in urine) [91, 92].

11. - Stromal cell-derived factor 1 (SDF-1)

Definition and pathway: SDF-1, also known as CXCL12, is a member of the CXC chemokine family considered a homeostasis/inflammatory molecule that participates in many physiological functions [93]. It is produced in multiple cell types (bone marrow, stromal cells, ECs…) and its receptors, chemokine receptor type 4 (CXCR-4) and CXCR-7, are widely distributed in the body. SDF-1/ CXCR-4 pathway is the most studied axis in this group, being implicated in cell proliferation, adhesion and migration, survival, and in the regulation of other pathways like PI3K/AKT (also involved in HHT pathogenesis) [68, 69]. Additionally, BMP-9 is an inducer of SDF-1 [94]. Hypoxia and inflammation are inducers of SDF-1 production, creating a concentration gradient that recruits mononuclear cells to the damaged place contributing to tissue repair and hypoxia-induced angiogenesis modulation via hypoxia-inducible factor-α (HIF1α) [94]. Dipeptidyl peptidase 4 (DPP4) acts removing SDF-1 amino terminal dipeptide and enhancing its cleavage, so mononuclear cells migration toward SDF1 gradient can be inhibited by DPP4 [95].

Evidence in HHT: Zucco *et al* observed that circulating angiogenic cells of HHT patients have a reduced migration towards VEGF and SDF1 [96]. Interestingly, mononuclear cells from HHT patients express elevated levels of DPP4 and might explain the disturbed homing toward damaged tissue [95]. In fact, the pretreatment of these cells with a DPP4 inhibitor has a beneficial effect on tissue repair and angiogenesis derived from the effect of SDF-1 pathway up-regulation [95]. Significantly, Wetzel-Strong *et al* showed significant higher levels of SDF1 in serum when comparing HHT patients to healthy controls [23].

Evidence in other vascular diseases: Patients with preeclampsia have elevated levels of SDF-1 and certain CXCR4 polymorphisms have been associated with this disease [97]. In 3.357 patients from the Framingham Heart Study, higher SDF-1 levels were associated with older age, lower levels of high-density lipoprotein-cholesterol and cigarette smoking; during follow-up (median 9.3 years) and after adjusting for clinical risk factors, higher SDF-1 levels were associated with new-onset HF and all-cause mortality [98]. Mehta *et al*, reported a prospective subanalysis of 3.687 patients with chronic kidney disease from the Chronic Renal Insufficiency Cohort Study; after a mean follow-up of 6 years and after adjusted for confounding factors, SDF-1 was associated with cardiovascular disease, mainly myocardial infarction [99].

In conclusion, biomarkers are a hot topic in vascular diseases as they can be used in daily clinical practice helping in diagnosis, risk stratification, prognosis or therapy monitoring. Additionally, they may be useful in pathogenesis understanding, and new therapeutic targets discovery. Angiogenic pathways are implied in multiple and common cardiovascular diseases like HF or atherosclerosis, but also in rare monogenic diseases like HHT. In this review, the most relevant potential angiogenic biomarkers with clinical impact in vascular diseases are discussed, aiming to help clinicians dealing with these patients and researchers to focus their investigations.

DECLARATIONS

Conflict of interest: the authors declare they have no conflict of interest.

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Availability of data and materials: Not applicable.

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References:

1. Lyngbakken MN, Myhre PL, Røsjø H, Omland T. Novel biomarkers of cardiovascular disease: Applications in clinical practice. Crit Rev Clin Lab Sci. 2019;56(1): 33-60.
2. Riera-Mestre A, Cerda P, Iriarte A, Graupera. Viñals M. Translational medicine in hereditary hemorrhagic telangiectasia. Eur J Intern Med. 2022;32–37.
3. Vasan RS. Biomarkers of cardiovascular disease: molecular basis and practical considerations. Circulation. 2006;113:2335–62.
4. The portal for rare diseases and orphan drugs. Available online: https://www.orpha.net/consor/cgi-bin/index.php (Accessed on Jul 4, 2020).
5. Faughnan ME, Mager JJ, Hetts SW, Palda VA, Lang-Robertson K, Buscarini E, et al. Second International Guidelines for the Diagnosis and Management of Hereditary Hemorrhagic Telangiectasia. Ann Intern Med. 2020;173: 989-1001.
6. Shovlin CL, Guttmacher AE, Buscarini E, Faughnan ME, Hyland RH, Westermann CJ, et al. Diagnostic criteria for hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome). Am J Med Genet. 2000;91: 66-7.
7. McDonald J, Wooderchak-Donahue W, VanSant Webb C, Whitehead K, Stevenson DA, Bayrak-Toydemir P. Hereditary hemorrhagic telangiectasia: Genetics and molecular diagnostics in a new era. Front Genet. 2015;5:1–9.
8. Sánchez-Martínez R, Iriarte A, Mora-Luján JM, Patier JL, López-Wolf D, Ojeda A, et al. Current HHT genetic overview in Spain and its phenotypic correlation: data from RiHHTa registry. Orpahnet J Rare Dis. 2020; 15:138.
9. Tillet E, Bailly S. Emerging roles of BMP9 and BMP10 in hereditary hemorrhagic telangiectasia. Front Genet. 2015;5:456.
10. Iriarte A, Ochoa-Callejero L, García-Sanmartín J, Cerdà P, Garrido P, Narro-Íñiguez J, et al. Adrenomedullin as a potential biomarker involved in patients with hereditary hemorrhagic telangiectasia. Eur J Intern Med. 2021;88:89–95.
11. Crist AM, Zhou X, Garai J, Lee AR, Thoele J, Ullmer C, et al. Angiopoietin-2 Inhibition Rescues Arteriovenous Malformation in a Smad4 Hereditary Hemorrhagic Telangiectasia Mouse Model. Circulation. 2019;139(17): 2049–2063.
12. Poole TJ, Finkelstein EB, Cox CM. The role of FGF and VEGF in angioblast induction and migration during vascular development. Dev Dyn. 2001;220(1):1-17.
13. Apte RS, Chen DS, Ferrara N. VEGF in Signaling and Disease: Beyond Discovery and Development. Cell. 2019;176(6):1248-1264.
14. Itatani Y, Kawada K, Yamamoto T, Sakai Y. Resistance to Anti-Angiogenic Therapy in Cancer-Alterations to Anti-VEGF Pathway. Int J Mol Sci. 2018;19(4):1232.
15. Robert F, Desroches-Castan A, Bailly S, Dupuis-Girod S, Feige JJ. Future treatments for hereditary hemorrhagic telangiectasia. Orphanet J Rare Dis. 2020;15(1):4.
16. Kazlauskas A. PDGFs and their receptors. Gene. 2017;614:1-7.
17. Melincovici CS, Boşca AB, Şuşman S, Mărginean M, Mihu C, Istrate M, et al. Vascular endothelial growth factor (VEGF) - key factor in normal and pathological angiogenesis. Rom J Morphol Embryol. 2018;59(2):455-467.
18. Adamis AP, Shima DT. The role of vascular endothelial growth factor in ocular health and disease. Retina. 2005;25(2):111-8.
19. Cirulli A, Liso A, D'Ovidio F, Mestice A, Pasculli G, Gallitelli M, et al. Vascular endothelial growth factor serum levels are elevated in patients with hereditary hemorrhagic telangiectasia. Acta Haematol. 2003;110(1):29-32.
20. Sadick H, Riedel F, Naim R, Goessler U, Hörmann K, Hafner M, et al. Patients with hereditary hemorrhagic telangiectasia have increased plasma levels of vascular endothelial growth factor and transforming growth factor-beta1 as well as high ALK1 tissue expression. Haematologica. 2005;90(6):818-28.

21. Sadick H, Hage J, Goessler U, Bran G, Riedel F, Bugert P, et al. Does the genotype of HHT patients with mutations of the ENG and ACVRL1 gene correlate to different expression levels of the angiogenic factor VEGF? Int J Mol Med. 2008;22:575–80.
22. Steineger J, Ueland T, Aukrust P, Michelsen A, Osnes T, Heimdal, et al. Pentraxin 3 level is elevated in hereditary hemorrhagic telangiectasia and reflects the severity of disease-associated epistaxis. Laryngoscope. 2019;129(1):E44-E49.
23. Wetzel-Strong SE, Weinsheimer S, Nelson J, Pawlikowska L, Clark D, Starr MD, et al. Pilot investigation of circulating angiogenic and inflammatory biomarkers associated with vascular malformations. Orphanet J Rare Dis. 2021;16(1):372.
24. Dupuis-Girod S, Ginon I, Saurin JC, Marion D, Guillot E, Decullier E, et al. Bevacizumab in patients with hereditary hemorrhagic telangiectasia and severe hepatic vascular malformations and high cardiac output. JAMA. 2012;307:948–55.
25. Liu DT, Frohne A, Koenighofer M, Frei K, Lucas T, Riss D, et al. Plasma VEGF - a candidate biomarker for response to treatment with bevacizumab in HHT patients. Rhinology. 2020;58(1):18-24.
26. Kaštelan S, Orešković I, Bišćan F, Kaštelan H, Gverović Antunica A. Inflammatory and angiogenic biomarkers in diabetic retinopathy. Biochem Med (Zagreb). 2020;30(3):030502.
27. Rana S, Burke SD, Karumanchi SA. Imbalances in circulating angiogenic factors in the pathophysiology of preeclampsia and related disorders. Am J Obstet Gynecol. 2022;226(2S):S1019-S1034.
28. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med. 2004;350(7):672-83.
29. Poon LC, Kametas NA, Maiz N, Akolekar R, Nicolaides KH. First-trimester prediction of hypertensive disorders in pregnancy. Hypertension. 2009;53:812–818. Am J Obstet Gynecol.
30. Ferroni P, Della-Morte D, Palmirotta R, Rundek T, Guadagni F, Roselli M. Angiogenesis and hypertension: the dual role of anti-hypertensive and anti-angiogenic therapies. Curr Vasc Pharmacol. 2012;10(4):479-93.
31. Botella LM, Albiñana V, Ojeda-Fernandez L, Recio-Poveda L, Bernabéu C. Research on potential biomarkers in hereditary hemorrhagic telangiectasia. Front Genet. 2015;6:115.
32. Ojeda-Fernandez L, Barrios L, Rodriguez-Barbero A, Recio-Poveda L, Bernabeu C, Botella LM. Reduced plasma levels of Ang-2 and sEng as novel biomarkers in hereditary hemorrhagic telangiectasia (HHT). Clin Chim Acta. 2010;411(7-8):494-9.
33. Akwii RG, Sajib MS, Zahra FT, Mikelis CM. Role of Angiopoietin-2 in Vascular Physiology and Pathophysiology. Cells. 2019;8(5):471.
34. Newman J, Brailovsky Y, Allen S, Bontekoe E, Masic D, Walenga J, et al. Angiopoietin-2 correlates with pulmonary embolism severity, right ventricular dysfunction, and intensive care unit admission. Vasc Med. 2021;26(5):556-560.
35. Huang S, Zhu Y, Ma Y, Qiu J, Sun Y, Mao Y, et al. Plasma Angiopoietin-2 as a Promising Biomarker for the Prognosis of Acute Pulmonary Embolism. Clin Lab. 2022;68(5).
36. Patel JV, Lim HS, Varughese GI, Hughes EA, Lip GY. Angiopoietin-2 levels as a biomarker of cardiovascular risk in patients with hypertension. Ann Med. 2008;40(3):215-22.
37. Höbaus C, Pesau G, Herz CT, Wrba T, Koppensteiner R, Schernthaner GH. Angiopoietin-2 and Survival in Peripheral Artery Disease Patients. Thromb Haemost. 2018;118(4):791-797.
38. Tsai YC, Lee CS, Chiu YW, Kuo HT, Lee CS, Hwang SJ, et al. Angiopoietin-2 as a Prognostic Biomarker of Major Adverse Cardiovascular Events and All-Cause Mortality in Chronic Kidney Disease. PLoS One. 2015;10(8):e0135181.

39. Pöss J, Ukena C, Kindermann I, Ehrlich P, Fuernau G, Ewen S et al. Angiopoietin-2 and outcome in patients with acute decompensated heart failure. Clin Res Cardiol. 2015;104(5):380-7.
40. Bolin M, Wiberg-Itzel E, Wikström AK, Goop M, Larsson A, Olovsson M et al. Angiopoietin-1/angiopoietin-2 ratio for prediction of preeclampsia. Am J Hypertens. 2009;22(8):891-5.
41. Medina-Jover F, Riera-Mestre A, Viñals F. Rethinking growth factors: the case of BMP9 during vessel maturation. Vasc Biol. 2022; 4(1):R1-R14.
42. Salmon RM, Jiang H, Morrell NW. Regulation of the ALK1 ligands, BMP9 and BMP10. Biochem Soc Trans. 2016;44(4): 1135-41.
43. Levet S, Ouarné M, Ciais D, Coutton C, Subileau M, Mallet C, et al. BMP9 and BMP10 are necessary for proper closure of the ductus arteriosus. PNAS. 2015;112 E3207–E3215.
44. Gallardo-Vara E, Tual-Chalot S, Botella LM, Arthur H, Bernabeu C. Soluble endoglin regulates expression of angiogenesis-related proteins and induction of arteriovenous malformations in a mouse model of hereditary hemorrhagic telangiectasia. Dis Model Mech. 2018;11(9):034397.
45. Desroches-Castan A, Tillet E, Bouvard C, Bailly S. BMP9 and BMP10: Two close vascular quiescence partners that stand out. Dev Dyn. 2022;251(1):178-197.
46. Kritharis A, Al-Samkari H, Kuter DJ. Hereditary hemorrhagic telangiectasia: diagnosis and management from the hematologist's perspective. Haematologica. 2018;103(9):1433-1443.
47. Hodgson J, Swietlik EM, Salmon RM, Hadinnapola C, Nikolic I, Wharton J, et al. Characterization of GDF2 Mutations and Levels of BMP9 and BMP10 in Pulmonary Arterial Hypertension. Am J Respir Crit Care Med. 2020;201(5):575-585.
48. Nikolic I, Yung LM, Yang P, Malhotra R, Paskin-Flerlage SD, Dinter T, et al. Bone Morphogenetic Protein 9 is a mechanistic Biomarker of Portopulmonary Hypertension. Am J Respir Crit Care Med. 2019;199(7):891-902.
49. Huang H, Wang W, Yang G, Zhang Y, Li X, Liu H, et al. Circulating bone morphogenetic protein-9 levels are associated with hypertension and insulin resistance in humans. J Am Soc Hypertens. 2018;12(5):372-380.
50. Xu X, Li X, Yang G, Li L, Hu W, Zhang L, et al. Circulating bone morphogenetic protein-9 in relation to metabolic syndrome and insulin resistance. Sci Rep. 2017;7(1):17529.
51. Luo Y, Li L, Xu X, Wu T, Yang M, Zhang C, et al. Decreased circulating BMP-9 levels in patients with Type 2 diabetes is a signature of insulin resistance. Clin Sci (Lond). 2017;131(3):239-246.
52 Castonguay R, Werner ED, Matthews RG, Presman E, Mulivor AW, Solban N, et al. Soluble Endoglin Specifically Binds Bone Morphogenetic Proteins 9 and 10 via Its Orphan Domain, Inhibits Blood Vessel Formation, and Suppresses Tumor Growth. J Biol Chem. 2011;286(34): 30034-46.
53. Kusanovic JP, Romero R, Chaiworapongsa T, Erez O, Mittal P, Vaisbuch E, et al. A prospective cohort study of the value of maternal plasma concentrations of angiogenic and anti-angiogenic factors in early pregnancy and midtrimester in the identification of patients destined to develop preeclampsia. J Matern Fetal Neonatal Med. 2009;22:1021–1038.
54. Vicen M, Igreja Sá IC, Tripská K, Vitverová B, Najmanová I, Eissazadeh S, et al. Membrane and soluble endoglin role in cardiovascular and metabolic disorders related to metabolic syndrome. Cell Mol Life Sci. 2021;78(6):2405-2418.
55. Malik RA, Li C, Aziz W, Olson JA, Vohra A, McHardy KC, et al. Elevated Plasma CD105 and Vitreous VEGF Levels in Diabetic Retinopathy. J Cell. Mol Med. 2005;9:692–7.
56. Kapur NK, Heffernan KS, Yunis AA, Parpos P, Kiernan MS, Sahasrabudhe NA, et al. Usefulness of soluble endoglin as a noninvasive measure of left ventricular filling pressure in heart failure. Am J Cardiol. 2010;106(12):1770–1776.

57. Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H, et al. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. Biochem Biophys Res Commun. 1993;192:553–60.
58. Koyama T, Ochoa-Callejero L, Sakurai T, Kamiyoshi A, Ichikawa-Shindo Y, Iinuma N, et al. Vascular endothelial adrenomedullin-RAMP2 system is essential for vascular integrity and organ homeostasis. Circulation. 2013;127:842-53.
59. Ochoa-Callejero L, Pozo-Rodrigálvarez A, Martínez-Murillo R, Martínez A. Lack of adrenomedullin in mouse endothelial cells results in defective angiogenesis, enhanced vascular permeability, less metastasis, and more brain damage. Sci Rep 2016;6:33495.
60. Iring A, Jin YJ, Albarrán-Juárez J, Siragusa M, Wang S, Dancs PT, et al. Shear stress-induced endothelial adrenomedullin signaling regulates vascular tone and blood pressure. J Clin Invest 2019;129:2775–91.
61. Hirano S, Imamura T, Matsuo T, Ishiyama Y, Kato J, Kitamura K, et al. Differential responses of circulating and tissue adrenomedullin and gene expression to volume overload. J Card Fail 2000;6: 120–9.
62. Lainchbury JG, Troughton RW, Lewis LK, Yandle TG, Richards AM, Nicholls MG. Hemodynamic, hormonal, and renal effects of short-term adrenomedullin infusion in healthy volunteers. J Clin Endocrinol Metab 2000;85:1016–20.
63. Kaafarani I, Fernandez-Sauze S, Berenguer C, Chinot O, Delfino C, Dussert C, et al. Targeting adrenomedullin receptors with systemic delivery of neutralizing antibodies inhibits tumor angiogenesis and suppresses growth of human tumor xenografts in mice. FASEB J 2009;23:3424–35.
64. Corti P, Young S, Chen CY, Patrick MJ, Rochon ER, Pekkan K et al. Interaction between alk1 and blood flow in the development of arteriovenous malformations. Development. 2011;138:1573-82.
65. Oh SP, Seki T, Goss KA, Imamura T, Yi Y, Donahoe PK, et al. Activin receptor-like kinase 1 modulates transforming growth factor-beta 1 signaling in the regulation of angiogenesis. Proc Natl Acad Sci USA. 2000;97:2626-31.
66. Mahmoud M, Allinson KR, Zhai Z, Oakenfull R, Ghandi P, Adams RH, et al. Pathogenesis of arteriovenous malformations in the absence of endoglin. Circ Res 2010;106:1425–33.
67. Kim W, Moon SO, Lee S, Sung MJ, Kim SH, Park SK. Adrenomedullin reduces VEGF-induced endothelial adhesion molecules and adhesiveness through a phosphatidylinositol 3′-kinase pathway. Arterioscler Thromb Vasc Biol 2003;23:1377–83.
68. Iriarte A, Figueras A, Cerdà P, Mora JM, Jucglà A, Penín R, et al. PI3K (Phosphatidylinositol 3-Kinase) activation and endothelial cell proliferation in patients with hemorrhagic hereditary telangiectasia type 1. Cells 2019;8:971.
69. Alsina-Sanchís E, García-Ibáñez Y, Figueiredo AM, Riera-Domingo C, Figueras A, Matias-Guiu X, et al. ALK1 loss results in vascular hyperplasia in mice and humans through PI3K activation. Arterioscler Thromb Vasc Biol 2018;38:1216–29.
70. Voors AA, Kremer D, Geven C, Ter Maaten JM, Struck J, Bergmann A, et al. Adrenomedullin in heart failure: pathophysiology and therapeutic application. Eur J Heart Fail. 2019;21(2):163-171.
71. Pandhi P, TerMaaten JM, Emmens JE, Struck J, Bergmann A, Cleland JG, et al. Clinical value of pre-discharge bio-adrenomedullin as a marker of residual congestion and high risk of heart failure hospital readmission. Eur J Heart Fail. 2020;22:683–91.
72. Martínez-Herrero S, Martínez A. Adrenomedullin: Not Just Another Gastrointestinal Peptide. Biomolecules. 2022;12(2):156.
73. Ristagno G, Fumagalli F, Bottazzi B, Mantovani A, Olivari D, Novelli D, et al. Pentraxin 3 in Cardiovascular Disease. Front Immunol. 2019;10:823.
74. Garlanda C, Bottazzi B, Magrini E, Inforzato A, Mantovani A. Ptx3, a Humoral pattern recognition molecule, in innate immunity, tissue repair, and cancer. Physiol Rev. 2018;98:623–39.

75. Suzuki S, Takeishi Y, Niizeki T, Koyama Y, Kitahara T, Sasaki T, et al. Pentraxin 3, a new marker for vascular inflammation, predicts adverse clinical outcomes in patients with heart failure. Am Heart J. 2008;155:75–81.
76. Matsubara J, Sugiyama S, Nozaki T, Akiyama E, Matsuzawa Y, Kurokawa H, et al. Incremental prognostic significance of the elevated levels of pentraxin 3 in patients with heart failure with normal left ventricular ejection fraction. J Am Heart Assoc. 2014;3:928
77. Colmenares-Mejía CC, Quintero-Lesmes DC, Bautista-Niño PK, Guio Mahecha E, Beltrán Avendaño M, Díaz Martínez LA, et al.
Pentraxin-3 is a candidate biomarker on the spectrum of severity from pre-eclampsia to HELLP syndrome: GenPE study. Hypertens Res. 2020;43(9):884-891
78. Klint P, Kanda S, Kloog Y, Claesson-Welsh L. Contribution of Src and Ras pathways in FGF-2 induced endothelial cell differentiation. Oncogene. 1999;3;18(22):3354-64.
79. Okada-Ban M, Thiery J, Jouanneau J. Fibroblast Growth Factor-2. The International Journal of Biochemistry & Cell Biology 32. 2000;263-267.
80. Choi E, Kim Y, Choe S, Tak YG, Garrido-Marin EM, Chang M, et al. Enhanced Responses to Angiogenic Cues Underlie the Pathogenesis of Hereditary Hemorrhagic Telangiectasia 2. PLoS ONE. 2013;8(5):e63138.
81. Song T, Wang W, Xu J, Zhao D, Dong Q, Li L, et al. Fibroblast Growth Factor 2 Inhibits Bone Morphogenetic Protein 9-Induced Osteogenic Differentiation of Mesenchymal Stem Cells by Repressing Smads Signaling and Subsequently Reducing Smads Dependent Up-Regulation of ALK1 and ALK2. Int J Biochem Cell Biol. 2013;45(8):1639-46.
82. El-Raggal NM, El-Farrash RA, Saad AA, Attia EAS, Saafan HA, Shaaban IS. Circulating Levels of Vascular Endothelial Growth Factor and Basic Fibroblastic Growth Factor in Infantile Hemangioma versus Vascular Malformations. Clin Appl Thromb Hemost. 2018;24(4):663-668.
83. Yang XJ, Jiang YH, Zheng JW, Hong L, Zhou Q, Qin ZP. The Role of Serum Basic Fibroblast Growth Factor, Estradiol and Urine Basic Fibroblast Growth Factor in Differentiating Infantile Haemangiomas from Vascular Malformations. Phlebology. 2011;26(5):191-6.
84. Haque S, Morris JC. Transforming growth factor-β: A therapeutic target for cancer. Hum Vaccin Immunother. 2017;13(8):1741-1750
85. Lenato GM, Guanti G. Hereditary Haemorrhagic Telangiectasia (HHT): Genetic and Molecular Aspects. Curr Pharma Des. 2006;12, 1173-1193.
86. Bloise E, Ciarmela P, Dela Cruz C, Luisi S, Petraglia F, Reis FM. Activin A in Mammalian Physiology. Physiol Rev. 2019;99(1):739-780.
87. Letarte M, McDonald ML, Li C, Kathirkamathamby K, Vera S, Pece-Barbara N, et al. Reduced endothelial secretion and plasma levels of transforming growth factor-beta1 in patients with hereditary hemorrhagic telangiectasia type 1. Cardiovasc Res. 2005;68(1):155–64.
88. Adu-Gyamfi EA, Lamptey J, Duan F, Wang YX, Ding YB. The transforming growth factor β superfamily as possible biomarkers of preeclmapsia: a comprehensive review. Biomark Med. 2019;13(15):1321-1330.
89. Yan Y, Wang XJ, Li SQ, Yang SH, LV ZC, Wang LT, et al. Elevated levels of plasma transforming growth factor-β1 in idiopathic and heritable pulmonary arterial hypertension. Int J Cardiol. 2016;222:368-374.
90. Goumans MJ, Ten Dijke P. TGF-β signaling in Control of Cardiovascular Function. Cold Spring Harb Perspect Biol. 2018;10(2):a022210.
91. Tsai YL, Chou RH, Kuo CS, Chang CC, Wu CH, Huang PH, et al. Circulating Activin A Is a Surrogate for the Incidence of Diastolic Dysfunction and Heart Failure in Patients With Preserved Ejection Fraction. Circ J. 2019:25;83(7):1514-1519.
92. Nagayama I, Maeshima A, Nagata D. Urinary Activin A: A Novel Biomarker for Human Acute Kidney Injury Diagnostics (Basel). 2022;12(3):661.

93. Li J, Chen H, Zhang D, Xie J, Zhou X. The role of stromal cell-derived factor 1 on cartilage development and disease. Osteoarthritis Cartilage. 2021;29(3):313-322.
94. Dingenouts CK, Goumans MJ, Bakker W. Mononuclear cells and vascular repair in HHT. Front Genet. 2015;6:114.
95. Post S, Smits AM, van den Broek AJ, Sluijter JP, Hoefer IE, Janssen BJ, et al. Impaired recruitment of HHT-1 mononuclear cells to the ischaemic heart is due to an altered CXCR4/CD26 balance. Cardiovasc Res. 2010;85(3):494–502.
96. Zucco L, Zhang Q, Kuliszewski MA, Kandic I, Faughnan ME, Stewart DJ, et al. Circulating angiogenic cell dysfunction in patients with hereditary hemorrhagic telangiectasia. PLoS One. 2014;9(2):e89927.
97. Karakus S, Bagci B, Bagci G, Sancakdar E, Yildiz C, Akkar O, et al. SDF-1/CXCL12 and CXCR4 gene variants and elevated serum SDF-1 levels are associated with preeclampsia. Hypertens Pregnancy. 2017;36(2):124-130.
98. Subramanian S, Liu C, Aviv A, Ho JE, Courchesne P, Muntendam P, et al. Stromal cell-derived factor 1 as a biomarker of heart failure and mortality risk. Arterioscler Thromb Vasc Biol. 2014;34(9):2100-5.
99. Mehta NN, Matthews GJ, Krishnamoorthy P, Shah R, McLaughlin C, Patel P, et al. Higher plasma CXCL12 levels predict incident myocardial infarction and death in chronic kidney disease: findings from the Chronic Renal Insufficiency Cohort study. Eur Heart J. 2014;35(31):2115-22.

Biomarker	Canonical pathway	Evidence in HHT	Evidence in other vascular diseases
VEGF and sVEGFR	Member of the PDGF family, binds to VEGFR2 in EC with proangiogenic effect [12, 13, 20]	Conflicting results: elevated versus normal plasma levels compared to healthy controls. Correlation with the response to nasal anti-VEGF therapy [19, 20, 21, 23]	Reduced in preeclampsia because of sVEGFR1 elevation, both predictors of the disease and related with worse prognosis [28]. Predictor of cardiovascular risk in hypertensive patients [30].
PlGF	Member of the PDGF family, binds to VEGFR2 replacing vascular VEGF-A, which is more available for VEGFR-2 enhancing angiogenesis [17].	No differences in HHT patients compared to healthy controls [22].	Elevated in preeclampsia and predictor of this disease in early stages and correlated to adverse maternal and perinatal outcomes [17].
ANGPT2	Proangiogenic glycoprotein of the Tie2/ANGPT pathway that works as an antagonist of ANGPT1. Produces destabilization of the vasculature and increases responsiveness to VEGF [11, 31]	Decreased levels in serum and EC of HHT patients, especially HHT2 and positive correlation between ANGPT2 and ESS and a negative one with hemoglobin levels [11].	Increased in most cardiovascular diseases [33], related to higher risk pulmonary embolism [34,35], predictor of cardiovascular risk in hypertensive, peripheral arterial disease and chronic kidney disease patients [36-38] and related to retinopathy in diabetic patients [33]. Also, increased levels predict worse prognosis at discharge in acute cardiac failure [39]. ANGPT1/ANGPT2 lower ratios predicts preeclampsia [40].
BMP9	Cytokine of the TGF-β superfamily, mainly produced by hepatocytes (BMP9) and heart (BMP10). It binds to TGFβR 1 and 2 and ALK1 [41].	Mutations related to HHT type 5. No differences between HHT patients and healthy controls [23, 46-47]	Reduced levels in patients with PoPH [48]. Decreased in relation to metabolic syndrome features [49-51]
sENG	Soluble extracellular domain of ENG, an auxiliary receptor for members of the TGF-β family. Anti-angiogenic profile in physiological situations and pro-inflammatory activity and contribution to endothelial dysfunction in pathological setting [44, 52].	Lower plasma levels in HHT1 and HHT2. There's a wider range of variation depending on the type of mutation, age and disease severity in HHT2 patients [23, 31, 44].	Higher levels as a predictor of preeclampsia and other placentation disorders [27, 53]. Higher levels related to cardiovascular risk and correlated with carotid intima-media thickness [54]. Increased in patients with diabetes, especially in those with retinopathy [55]. Biomarker of cardiac failure [56].

AM	Peptide hormone from the amylin/calcitonin gene-related family secreted by EC and smooth muscle cells. Binds to calcitonin receptor-like receptor (CLR) and AM1 and AM2 receptors and directly stimulates angiogenesis producing vasodilatation and regulating vascular stability and permeability [57-60].	Contribute to development of VMs [64-65]. Higher AM serum levels in HHT patients and in telangiectasia biopsies [66].	Biomarker of fluid overload in HF [64, 72]. Increased in diabetic microangiopathy [72].
PTX3	Acute-phase response protein of the long pentraxin group. It is produced at the site of the lesion acting as a pattern recognition molecule and contributing to tissue repair. [73, 74].	Increased in HHT patients compared to healthy controls and correlated with the ESS, blood transfusions and hemoglobin [22].	Predictor of all-cause mortality after myocardial infarct when it's raised [73]. Predictor of adverse outcomes in HF when is raised [75-76]. Correlation with preeclampsia when is raised [77].
FGF2	Ubiquitously expressed, acts in both autocrine and paracrine manners interacting with FGFR1 and inducting cell proliferation and differentiation. Promotes angiogenesis by itself but also boosting VEGF effect [78, 79].	ALK1 inhibits FGF2-induced angiogenesis [84]. It might impair BMP9 signaling in ECs and trigger vascular lesions after a second hit event in HHT patients [80, 81].	FGF2 serum levels are increased children with either VMs or hemangiomas [82, 83].
TGF-β	Member of the TGF-β super family of cytokines that acts as a powerful angiogenic factor and a mediator of vascular remodeling [84, 85]	Controversial results in serum levels of HHT patients [20, 22, 23, 87]. Correlation of serum levels with the number of blood transfusions and the ESS [22].	Conflicting results between TGF-β and preeclampsia [88]. Raised in idiopathic and heritable pulmonary hypertension [89]. Related to advanced stable atherosclerotic disease [90].
Activin A	Member of the TGF-β super family of cytokines that mediates major cell proliferation, differentiation, metabolism, repair, and apoptosis [86]	Not found any differences in serum levels between HHT patients and healthy controls [22].	Raised as a predictor of preeclampsia [88]. Raised Activin A is related to worse prognosis in diastolic dysfunction HF or acute kidney failure [91, 92].
SDF-1	Homeostasis/inflammatory molecule member of the CXC chemokine family,	Elevated levels of SDF1 in serum when comparing HHT patients to healthy controls	Elevated levels of SDF-1 in preeclampsia [97]. Higher levels in older patients with low HDL

	binds to CXCR-4 and 7 and participates in many physiological functions and in inflammatory settings [93].	[23].	cholesterol levels, smokers and associated with all-cause mortality and new-onset HF [98]. Associated with cardiovascular disease development [99].

Table 1: Biomarkers pathways and evidence in HHT and other vascular diseases.

Abbreviations: ALK-1: Activin receptor-like kinase 1; AM: adrenomedullin; ANGPT: angiopoietin; AVM: arterio-venous malformation; BMP: Bone morphogenetic protein; CXCR: chemokine receptor; EC: endothelial cells; ESS: epistaxis severity score; FGF2: fibroblast growth factor 2; FGFR1: FGF receptor 1; HDL: high-density lipoprotein; HF: heart failure; HHT; hereditary hemorrhagic telangiectasia; PDGF: Platelet-derived growth factor; PlGF: placental growth factor; PoPH: portopulmonary hypertension; PTX: pentraxin 3; sENG: soluble endoglin; SDF-1: stromal cell derived factor 1; TGF- β: transforming growth factor β; VEGF: vascular endothelial growth factor; VEGFR: VEGF receptor; VM: vascular malformation.

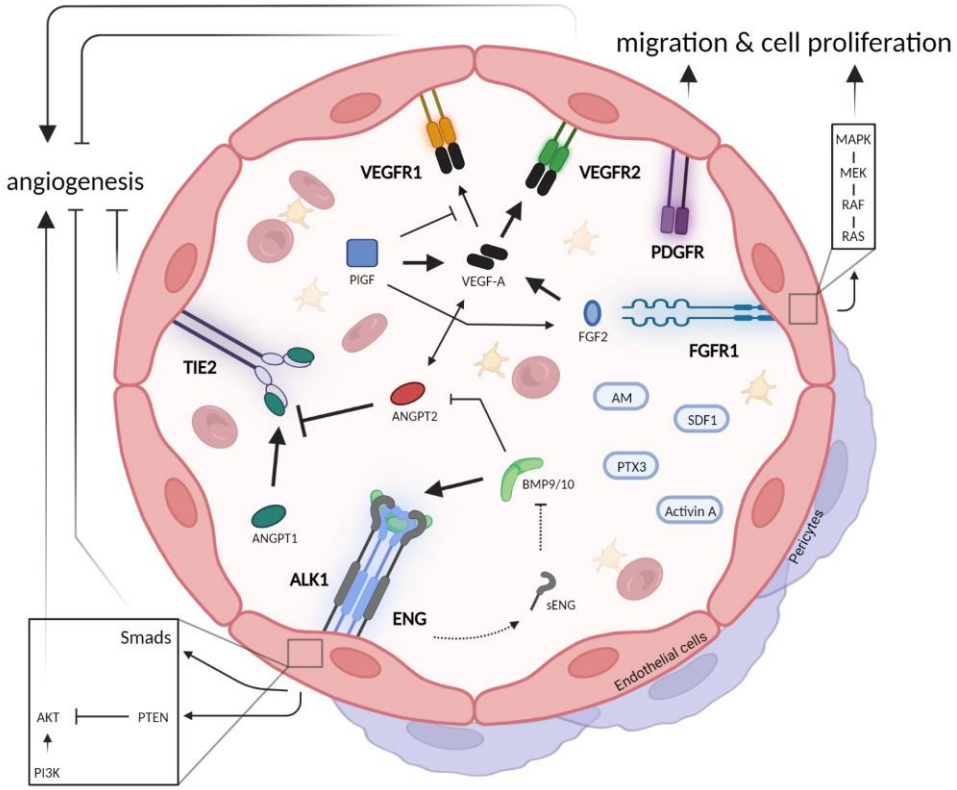
migration & cell proliferation
angiogenesis
VEGFR1
VEGFR2
PDGFR
MAPK
MEK
RAF
RAS
PlGF
VEGF-A
FGF2
FGFR1
TIE2
ANGPT2
AM
SDF1
PTX3
BMP9/10
Activin A
ANGPT1
ALK1
ENG
sENG
Pericytes
Endothelial cells
Smads
AKT
PTEN
PI3K

Figure 1: Summary of angiogenesis related cells and pathways and how they interact. Thicker lines are canonical functions while thinner lines express less intense mechanisms. Arrow ending means activation while plain ending means inhibition. Double arrow means interaction without explicit activation/inhibition role. Dashed lines are suggested mechanisms. Red cells in the lumen are erythrocytes while the yellows are platelets. Pathways depicted in squares are meant to happen in the cytosol of endothelial cells.

Abbreviations: ALK1, activin receptor-like kinase 1; AM, adrenomedullin; FGF2, fibroblast growth factor type 2; FGFR1, fibroblast growth factor receptor 1; PDGFR, platelet derived growth factor receptor; PlGF, platelet growth factor; PTX3, pentraxin 3; SDF1, Stromal cell-derived factor 1; sENG, soluble Endoglin; VEGF-A, vascular endothelial growth factor type A; VEGFR1, vascular endothelial growth factor 1; VEFGR2, vascular endothelial growth factor 2.

HIGHLIGHTS:

- Angiogenic pathways are implied in multiple cardiovascular diseases
- The evidence of angiogenic biomarkers for managing vascular diseases is scarce
- Here, the most relevant potential angiogenic biomarkers with clinical impact in vascular diseases are discussed
- Hereditary hemorrhagic telangiectasia (HHT) is used as a model of vascular disease with angiogenic disturbances
- This information will help clinicians dealing with these patients and researchers to focus their investigations