Vascular growth factors as potential new treatment in cardiorenal syndrome in diabetes

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Abstract

Background: Cardiorenal syndrome in diabetes is characterised by alterations of the cardiovascular system paralleled by kidney disease with progressive renal function decline. In diabetes, chronic metabolic and haemodynamic perturbations drive endothelial dysfunction, inflammation, oxidative stress and progressive tissue fibrosis which, in turn, lead to heart and renal anatomo-functional damage. In physiology, vascular growth factors have been implicated in vascular homeostasis; their imbalance, in disease setting such as diabetes, leads to vascular dysfunction and cardiorenal damage.

Aims: To define the role of vascular growth factors and angiopoietins in cardiorenal syndrome.

Material and Methods: We will focus on the two most studied vascular growth factors, vascular endothelial growth factor (VEGF) and angiopoietins (Angpt). The balance and crosstalk between these growth factors are important in organ development and in the maintenance of a healthy vasculature, heart and kidney. The observed alterations in expression/function of these vascular growth factors, as seen in diabetes, are a protective response against external perturbations.

Results: The chronic insults driving diabetes-mediated cardiorenal damage results in a paradoxical situation, whereby the vascular growth factors imbalance becomes a mechanism of disease. Studies have explored the possibility of modulating the expression/action of vascular growth factors to improve disease outcome. Experimental work has been conducted in animals and has been gradually translated in humans.

Discussion: Difficulties have been encountered especially when considering the magnitude, timing and duration of interventions targeting a selective vascular growth factor. Targeting VEGF in cardiovascular disease has been challenging, while modulation of the Angpt system seems more promising.

Conclusion: Future studies will establish the translatability of therapies targeting vascular growth factors for heart and kidney disease in patients with diabetes.

Keywords
cardiorenal syndrome, endothelial dysfunction, fibrosis, inflammation, vascular growth factors
1 | CARDIORENAAL SYNDROME

The cardiorenal syndromes (CRSs) result following an acute and/or chronic insult to either the heart or the kidney and manifest with a generalised anatomo-functional dysfunction of the heart/vasculature and renal systems. CRSs encompass a variety of pathophysiological mechanisms such as chronic inflammation, imbalance in reactive oxygen species/nitric oxide production, chronic renin-angiotensin aldosterone system (RAAS) activation, endothelial dysfunction (ED) and, ultimately, tissue fibrosis (Figure 1).

Diabetes is the most diffuse and important clinical condition driving CRS and is characterised by an established cardiovascular disease where a significant proportion of patients eventually develop kidney disease. ED and tissue fibrosis, respectively, the initial and latest manifestation of CRS are observed in the diabetic heart and kidney and drive the significant clinical burden of this disease.

2 | ENDOTHELIAL DYSFUNCTION

Endothelial dysfunction is a condition of the endothelium characterised by a reduction in production and/or availability of nitric oxide (NO) and alteration in endothelium-dependent vasodilation, known as the ability of the endothelium to synthesise the paracrine factor endothelium-derived relaxing factor, potent vasodilating agent acting as inhibition of vascular smooth muscle cells contraction.

Endothelial dysfunction (Figure 2) is characterised by increased vascular permeability, complement activation and stimulation of a proinflammatory and prothrombic state paralleled by a systemic alteration in vascular wall structure (eg loss of endothelial glyocalyx, endothelial cell apoptosis, alteration in the basement membrane structure). ED affects also the outer layer of the vascular wall, whereby the vascular smooth muscle cell phenotype switches from the ‘contractile’ to the ‘synthetic’ state resulting in an increase in cell proliferation and synthesis of extracellular matrix (ECM).

In CRSs, diabetes-mediated ED is responsible for the activation of a series of mechanisms that lead to vascular dysfunction in both the renal and coronary (heart) circulation. Classical risk factors for the development of ED are age, male gender, dyslipidaemia, hypertension and poor lifestyle such as smoking and lack of physical activity.

Morphological studies on the kidney, heart and aorta have shown profound ED-mediated pathological changes, manifested by disorganisation of the vascular cells lining with structural and functional alterations of the endothelium, subendothelial layer and basement membrane. The major mechanisms driving ED in diabetes are the accumulation of free radicals secondary to activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase by angiotensin-2 and endothelin-1, to an increase in mitochondrial superoxide and to the uncoupling of endothelial NO synthase (eNOS) where synthesis of reactive oxygen species (ROS) substitutes NO production.

Increased production of free radicals reacts with vasoactive substances and impair vascular responsiveness to vasodilating agents. Indeed, increase in superoxide leads to the spontaneous formation of peroxynitrite (a strong oxidant), an important mediator of vascular damage.

The described protective role of antioxidants in experimental models indicates that increased ROS production and low NO bioavailability are responsible for the impaired vasorelaxation.

It is worth noting that ROS are important signalling molecules for growth factor-mediated vascular physiological...
Endothelial dysfunction is a major determinant of tissue fibrosis in CRS. Endothelial dysfunction and progressive tissue fibrosis represent an unifying common mechanism at the basis of CRS in diabetes.

responses\textsuperscript{20,21}, as an example, ROS derived from NADPH oxidase are important messengers mediating the vascular endothelial growth factor (VEGF) receptor 2 (VEGFR2)/KDR receptor signalling system, an important cellular pathway for vascular stability\textsuperscript{22}. This suggests that antioxidant therapy, when implemented, will have to be tuned in order to maintain a physiological level of cellular ROS.

Endothelium stability, in physiology and in the repair process as seen in diseases, is the result of an equilibrium among pro- and anti-angiogenic factors; imbalance in the expression of angiogenic factors leads to an impaired vascular remodelling/repair, which alters vessel wall stability and contributes to disease manifestation\textsuperscript{23}.

Below, we will discuss the role of the major vascular growth factors which have been implicated in vascular dysfunction in diabetes and their potential future translation in clinical practice.

2.1 | Role of vascular growth factors in the diabetic heart and kidney

Vascular growth factors play both a pivotal role in endothelial function in physiology and in the pathophysiology of CRS in diabetes. The VEGF/VEGFR and Angpt/Tie receptor systems have been extensively studied in diabetes.

2.2 | The vascular endothelial growth factor/VEGFR system

Vascular endothelial growth factors and their receptors are important in vascular development and in vascular remodelling and repair in disease conditions. VEGFs, a dimeric molecule, exist in five different isoforms (VEGFA, B, C, D and Placental growth factor). VEGFs proteins bind to a class of tyrosine kinases receptors VEGFs’ receptors (VEGFR) 1, 2 and 3 that mediate their biological effects\textsuperscript{24}. VEGFRs are highly expressed in different cells and tissues predominantly in macrophages, endothelium and smooth muscle cells in the vasculature. VEGFR1, or Flt1, is involved in vascular development during embryogenesis, in wound healing and monocyte migration. Both VEGFA and B bind VEGFR1. VEGFR2, or Flk1, is the principal receptor involved in angiogenesis and vascular remodelling and specifically endothelial cell migration and survival, mitogenesis and vascular permeability. VEGFR2 interacts with VEGFA, VEGFC and VEGFD. Lastly, VEGFR3, activated by VEGFC and VEGFD, is mainly thought to be involved in lymphatic vessel formation, with some studies associating this receptor with tumour angiogenesis\textsuperscript{25}.

In physiology, VEGFA fulfils its effects by promoting endothelial cell survival and the synthesis of vasodilatory mediators. The VEGFA/VEGFR2 signalling system increases eNOS activity and NO production promoting vasodilation\textsuperscript{26}, and through the activation of phosphoinositide 3-kinase (PI3K)/Akt pathway promotes vascular permeability\textsuperscript{27,28}. Further, VEGFA signalling stimulates the production of the vasodilatory prostanooid prostacyclin through activation of phospholipase-A2 via PLCγ/PKC\textsuperscript{29} and antagonises the contractive effect of endothelin-1\textsuperscript{30}.

In chronic disease conditions, as seen in diabetes, the beneficial actions of VEGFA are lost, and this growth factor, from protective as seen in physiology, becomes one of the main drivers of ED and vascular disease.

Sustained metabolic and haemodynamic perturbations, as seen in diabetes, drive a sustained increase in mitochondrial superoxide and activation of NADPH oxidase. The secondary increase in ROS results in eNOS uncoupling which, in turns, contributes to the increase in cellular oxidative stress\textsuperscript{15}.

VEGFA becomes a driver of free radical accumulation, promoter inflammation and upregulation of inflammatory cytokines (eg tumour necrosis factor-α and interleukin-6)\textsuperscript{31,32}. 
The increase in VEGFA, in a setting of increased oxidative stress/inflammation, promotes impaired vascular remodelling which results in an abnormal vascular repair process with leaky and thin new vessels. VEGFB is highly expressed in the heart (as in metabolically active tissues) and modulate metabolic processes such as trans-endothelial transport of circulating fatty acids for myocytes uptake and oxidation.

VEGFB also favours the expression of metalloproteinase inhibitor 1, that by forming a one to one complex with specific metalloproteinases (MMP)(eg collagenases), and results in MMP inactivation favouring ECM deposition.

Despite VEGF is not the major VEGF isoform expressed in the heart, its action in the heart vasculature, in the setting of diabetes, could result in chronic activation of inflammation, oxidative stress and lead to ischaemic coronary artery disease and progressively evolve towards diabetic cardiomyopathy.

VEGFB, the most abundant isoform in the normal heart, has two isoforms: VEGFB186 and VEGFB167, with the latter representing approximately 80% of the total VEGFB transcripts. VEGFC and VEGFD isoforms are induced in condition of heart failure.

VEGFB is highly expressed in the heart (as in metabolically active tissues) and modulate metabolic processes such as trans-endothelial transport of circulating fatty acids for myocytes uptake and oxidation.

VEGFB levels are decreased in experimental animal models and humans with heart failure. Despite suggestion for a protective role of VEGFB in the heart, its role in diabetic cardiomyopathy is unknown. Recently, it has been observed that, in experimental animal models, hyperglycaemia, as seen in diabetes, is paralleled by a decrease in VEGFB expression and action, and this could lead to cardiac dysfunction as seen in diabetes.

A close interplay exists between endothelial cells, expressing heparinase, to release the surface-bound VEGFB on cardiomyocytes to promote cell survival and protecting against development and progression of cardiomyopathy in diabetes.

Indeed, downregulation of VEGF levels in the heart has been proposed as one of the factors driving the increased risk in cardiovascular morbidity and mortality in patients with diabetes.

Conversely to the heart, VEGFA plays a key role in the early phase of diabetic kidney disease. VEGFA is constitutively expressed in the glomerular podocytes contributing to its physiological role. In diabetes, VEGFA is upregulated both in the glomeruli (podocytes, endothelial cells, mesangial cells) and in the tubular compartment and, secondary to chronic haemodynamic/metabolic perturbation and eNOS uncoupling, drives altered vascular remodelling, inflammatory processes and ultimately glomerulosclerosis and tubulointerstitial fibrosis. Indeed, in experimental animal models for diabetes, inhibition of VEGFA or its receptor VEGFR2 results in amelioration of diabetic kidney disease and its overexpression results in increase fibrosis.

As chronic kidney disease progresses, VEGFA glomerular expression falls as the glomerular tuft is progressively substituted by deposition of ECM (glomerulosclerosis).

Complete inhibition of VEGF in the kidney results in endothelial apoptosis and proteinuria, making VEGF an important survival growth factor for the vasculature.

### 2.3 The Angiopoietin Tie2/Tie1 receptor system

Angiopoietins (Angpt), with Angpt1 and Angpt2 as the two major isoforms, are a class of vascular growth factors that play an important role on vascular development and postnatal angiogenesis.

In physiology, Angpt1 signals via the Tie2 receptor, on endothelial cells, and promotes endothelial cell survival, vascular stability and maturation via inhibition of the transcription factor forkhead box O1 (FOXO1) (Figure 3).

Conversely, Angpt2, by competing with the Angpt1 binding to the Tie2 receptor, inhibits Angpt1-mediated Tie2 receptor (phosphorylation) activation.

Tie1, an orphan receptor of the Tie family, interacts with Tie2, and although it apparently does not bind directly to Angpt1/2, it can modulate the Angpt1/2 effect when bound to Tie2.

Tie1 potentiates signalling at the Tie2 receptor under normal conditions by forming a complex with Tie2. Conversely, in disease conditions such as inflammation, when Tie1 is cleaved, the Angpt1-mediated activation of Tie2 is downregulated.

Angpt2 can act as both an agonist and an antagonist at the Tie2 receptor: in the presence of Tie1, as in physiological conditions, Angpt2 acts as a weak agonist of Tie2; in disease, when Tie1 receptor is downregulated, Angpt2 acts as an inhibitor of Tie2 receptor that results in destabilisation of the vessel wall (Figure 3).

Angpt1 induces arteriolar vasodilation, via release of NO through eNOS stimulation, and prevents vascular leakage...
through regulating vascular endothelial adhesion molecule (VE)-cadherin phosphorylation suggesting that Angpt1 is an important regulator of microvascular tone and vascular stabilisation51,52.

Angpt1 inhibits the VEGFA-mediated phosphorylation-dependent redistribution of VE-cadherin promoting an intact, less permeable, endothelial barrier and promotes the association of mDia/Src which results in deprivation of essential molecules required for VEGFR-mediated paracellular vascular permeability53.

In turn, VEGFA can promote proteolytic cleavage and shedding of Tie2 receptor via PI3K/Akt-dependent pathway and results in vascular wall destabilisation and stimulation of angiogenesis and vascular remodelling44.

Angpt2 is an independent predictor of adverse cardio renal clinical outcomes in patient with diabetes55. Further, Angpt2 is also known as an unfavourable outcome marker in patients with ischaemic heart disease56 and heart failure57.

Overexpression of Angpt2 in the heart, in experimental animal model of diabetes, stimulates inflammation, endothelial apoptosis and accumulation of ECM with myocardial fibrosis58. In experimental animal model of heart ischaemia, Angpt2 is highly expressed in endothelial cells at the border of the ischaemic myocardium and worsen cardiac hypoxia and inflammation after myocardial ischaemia59.

An increased Angpt2/Angpt1 ratio in the myocardium favours inflammation, endothelium apoptosis with loss of endothelial glycocalyx and increased ECM deposition with alteration of basement membrane and vascular leakage59.

Reversal of Angpt2/Angpt1 imbalance in favour of Angpt1 ameliorates the post-ischaemic vascular remodelling60 and therefore a decrease in Angpt2/Angpt1 ratio by either inhibition of Angpt2 or overexpression of Angpt1 could be therapeutic option for ischaemic heart disease and heart failure.

The imbalance towards an increased Angpt2/Angpt1 ratio is also evident in patient with chronic kidney disease (CKD) stage 3-561, a condition closely linked with poor cardiovascular outcome62.

An imbalance of Angpt1/2, with decreased levels of Angpt1 and increased levels of Angpt2, is strongly associated with degree of renal impairment and albuminuria63.

In humans and in experimental in vitro and in vivo models, VEGFA and Angpt2 are upregulated in the early stages of diabetic glomerulopathy, while Angpt1 is downregulated64-66; this milieu is associated with altered vascular remodelling, endothelial proliferation and increased capillary permeability31,41.

Podocyte (glomerular)-specific upregulation of Angpt2, in healthy adult mice, is paralleled by an increase in albuminuria, VEGFA/VEGFR2 system downregulation and endothelial cells apoptosis67. Similarly, podocyte-specific overexpression of Angpt1 ameliorates diabetes glomerulosclerosis and proteinuria65.
The Angpt1-2/Tie1-2 receptor system is important in both the heart and the kidney both in physiological and disease setting, and a correction of the Angpt1/2 imbalance in favour of Angpt1 could represent a potential treatment target. Further, overexpression or prevention of Tie1 shedding could represent another potential therapeutic approach to maintain a sustained Tie2 receptor activation in disease setting.

Similarly to VEGF, studies are warranted to dissect the magnitude and timing of the intervention to confer benefit in condition of heart and kidney disease.

### 2.4 Fibrosis in CRSs

Accumulation of ECM and progressive heart and kidney fibrosis in diabetes is the final ‘irreversible’ event that leads to cardiac and renal dysfunction and organ death.

Heart and kidneys interstitium are dynamic structures that undergo a continue process of deposition and degradation of ECM. When the deposition of ECM overcomes its degradation, tissue fibrosis occurs and leads, in the heart, to reduced ejection fraction and impaired electric conductance with fibrosis of the myocardium and, in the kidney, to a relentless and progressive reduction in renal function with glomerulosclerosis and tubulointerstitial fibrosis.

Epithelial and endothelial to mesenchymal transition (EMT, EndoMT), a reversible cellular event whereby epithelial or endothelial cells differentiate towards a mesenchymal phenotype, contributes significantly to the fibrotic process. In EMT/EndoMT, epithelial and endothelial cells progressively lose their normal function and characteristic appearance and transform into spindle-shaped cells with fibrosis of the myocardium and, in the kidney, to a relentless and progressive reduction in renal function with glomerulosclerosis and tubulointerstitial fibrosis.

Expression of the genes involved in EndMT is decreased in the heart endothelial cell of mice with endothelial-specific deletion of Angpt2, implicating Angpt2 and inhibition of Tie2 activation in EndMT. Specific direct role of angiopoietin system on EMT/EndoMT has not been directly investigated.

### 2.5 Targeting vascular growth factors in CRS

(Table 1) Vascular growth factors are important for the maintenance of a healthy endothelium and correction of the imbalance in their expression in disease setting could potentially open new avenues for treatment of disease progression in both the heart and kidney.

In parallel to the attempts in correcting the primary noxae (metabolic and haemodynamic perturbations) driving cardio-renal disease in diabetes, parallel research has proposed that correction of the vascular growth factors ‘imbalance’ could represent a parallel promising therapeutic strategy.

Anti-VEGFA therapy could represent a potential treatment for diabetic kidney disease but the use of VEGFA inhibitors in diabetic retinopathy and in neoplastic disease has shown that an excessive inhibition of VEGFA could result in proteinuria. Studies have highlighted the importance of constitutive VEGF expression in glomerular physiology, and therefore, any attempt for a therapeutic inhibition of the VEGFA/VEGFR2 system in diabetes should account for a sufficient residual function of the VEGFA/VEGFR2 system.

Conversely to what is seen in the kidney VEGF is beneficial for the heart as inhibitors of VEGF signalling utilised for treatment of cancer or angioproliferative eye disease have been linked with cardiovascular toxicity.

Most inhibitors of VEGF signalling result in an increase in blood pressure, cardiac ischaemia, arterial thromboembolism and cardiac dysfunction. This highlights the importance of VEGF in vascular homeostasis, and the consequence of its inhibition leads to endothelial cell apoptosis and other adverse events such as vascular wall and plaque instability and arterial thrombosis as reported in experimental animal models.

In diabetes, the progressive decline in cardiac function and parallel myocardial fibrosis is associated with a decrease in capillary density and cardiac blood supply, and in diabetic rodents, VEGFA therapy is able to confer a restoration of microvasculature and recovery of cardiac function. Modulation of angiogenesis with VEGF inhibition could be potentially harmful especially in diabetes, a condition characterised by a reduction in the vascular reserve (diabetic cardiomyopathy), and lead to cardiotoxicity.

Diabetic cardiomyopathy is a complex disease, and ED represents a primary mechanism of its pathophysiology. In
diabetes, upregulation of VEGFA drives vascular damage, as VEGFA is not highly expressed in the heart, its targeting in the myocardium is debatable.

Early work, in the heart, has highlighted a potential protective role for VEGFB overexpression. In experimental animal model, overexpression of VEGFB gene (both VEGFB186 and VEGFB167 isoform) induces physiologic heart hypertrophy and cardio- protection after myocardial infarction with VEGFB favouring heart vascularisation.

VEGFC/VEGFR3 axis mediates TGFβ1-induced epithelial-to-mesenchymal transition and is upregulated in heart failure. Targeting VEGFC could be important to reduce fibrosis.

More work needs to be done to elucidate the exact role of the different isofrom of VEGF in diabetic cardiomyopathy and targeting of VEGF action is premature.

Modulation of the Angpt1/Tie2 receptor system with inhibition of the vascular endothelial-protein tyrosine phosphatase (VE-PTP) results in sustained Tie2 activation and inhibition of vascular permeability and new vessel formation. Use of VE-PTP inhibitors has led to promising results, both in experimental animal models and in patients with diabetic macular oedema. In experimental animal model of diabetic kidney disease, genetic deletion of VE-PTP resulted in activation of eNOS and inhibition of FOXO1 transcription.

**TABLE 1** Synopsis of VEGF- and ANGPT role in cardiorenal syndrome in diabetes

|                  | Heart disease in diabetes | Kidney disease in diabetes |
|------------------|---------------------------|---------------------------|
| VEGFA            | Main driver of:           | Main drivers of:          |
|                  | • endothelial dysfunction | • endothelial dysfunction |
|                  | • vascular inflammation   | • vascular inflammation   |
|                  | • vascular permeability   | • vascular permeability   |
| VEGFB            | Promoter of:              |                           |
|                  | • cell survival           |                           |
|                  | • antiapoptotic           |                           |
| VEGF targeting effect | • Putative protective role for VEGFA modulation/inhibition | • Putative protective role for VEGFA modulation/inhibition |
|                  | • Putative protective role for VEGFB overexpression |                           |
| Current barriers for treatment | Issues to be resolved: | Issues to be resolved: |
|                  | • Timing during disease stages | • Timing during disease stages |
|                  | • Magnitude of VEGFA inhibition | • Magnitude of VEGFA inhibition (excessive VEGFA inhibition can result in proteinuria, hypertension, renal thrombotic microangiopathy) |
|                  | • Magnitude of VEGFB stimulation |                           |
| ANGPT1           | Promoter of:              | Promoter of:              |
|                  | • Vascular stability      | • Vascular stability      |
|                  | • Cell survival           | • Cell survival           |
|                  | • anti-inflammatory       | • anti-inflammatory       |
|                  | • post-ischaemic vascular remodelling | • vascular repair/remodelling |
| ANGPT2           | Promoter of:              | Promoter of:              |
|                  | • Inflammation            | • Inflammation            |
|                  | • endothelial cells apoptosis | • endothelial apoptosis |
|                  | • vascular permeability   | • vascular permeability (albuminuria) |
|                  | • fibrosis                | • fibrosis                |
| ANGPT targeting effect | • Angpt1 signalling improves vascular repair/remodelling and inhibit fibrosis | • Angpt1 signalling promotes vascular health and inhibit vascular permeability |
|                  | • Organ protection        | • Organ protection        |
| Current barriers for treatment | Issues to be resolved: | Issues to be resolved: |
|                  | • Timing during disease stages | • Timing during disease stages |
|                  | • Magnitude of Angpt1 stimulation/Angpt2 inhibition | • Magnitude of Angpt1 stimulation/Angpt2 inhibition |
factor, with reduction of proinflammatory and profibrotic gene expression and secondary renal protection\textsuperscript{89}. Inhibition of hyperglycaemia-mediated upregulation of PTP in diabetic db/db mouse enhances Angpt1/Tie2 signalling and improves angiogenesis in the diabetic heart suggesting that restoration of Angpt1/Tie2 signalling by PTP inhibitors is a promising avenue to translate in the clinical setting\textsuperscript{90}.

Overexpression of Angpt2 in the db/db mouse heart inhibited Tie2 and VEGF expression resulting in cell apoptosis and interstitial fibrosis formation with parallel loss in capillary density\textsuperscript{58}. Conversely, overexpression of Angpt1 reversed loss of capillaries and fibrosis in db/db mouse hearts\textsuperscript{60}. As both Angpt and VEGFA drive, in disease setting, the expression of TGFβ1, in the kidney and the heart\textsuperscript{70,91} targeting of TGFβ has been proposed to antagonise fibrotic disorders. In experimental animal models of diabetes, inhibition of TGFβ1 signalling ameliorated renal fibrosis\textsuperscript{92}. Conversely, TGFβ1 neutralising antibodies failed to demonstrate renoprotection patient with diabetic kidney disease\textsuperscript{93}. TGFβ1 regulates multiple physiological cellular function, and studies should aim at selective inhibition of the TGFβ1-mediated profibrotic actions\textsuperscript{94}.

TGFβ is also implicated in cardiac fibrosis and cardiac hypertrophy. TGFβ levels are elevated in myocardial infarct and worsen myocardial injury. Therapeutic targeting of TGFβ signalling has had limited success in experimental model of cardiac dysfunction and heart failure, and more research is needed to develop therapies targeting solely the profibrotic arm of the TGFβ signalling pathway\textsuperscript{95}.

\section*{3 | CONCLUSIONS}

The dysregulated VEGF and Angpt response seen in the diabetic heart and kidney has primed a lot of research to suggest potential newly targetable pathways in the treatment of cardiorenal disease in diabetes. Many lessons have been learned on what to target (eg different vascular growth factors isoforms, receptors), on the importance of timing of when a specific treatment should be started in respect to the evolution and progression of cardiorenal disease, and for how long.

Specifically, the important concept learned is that modulation of a biological process should be preferred to its complete inhibition or activation. Further, that modulation of vascular growth factors should be adjusted depending on disease stage (Figure 4).

There is clearly a lot of hesitation around the specific targeting of VEGF pathways as they seem to differ between the heart and the kidney.

It is our impression that modulation of the Angpt1-2/Tie1-2 receptor system could be easier to translate to the clinic as studies have provided more clear-cut answers. Importantly, because of the close crosstalk between the Angpt and VEGF system we expect some parallel beneficial modulation of VEGF signalling by targeting the Angpt/Tie system.

In the last 15-20 years, many advances have been made on the vascular growth factor systems and the future might reserve some positive new discoveries for the treatment of cardiorenal disease in patients with diabetes.

\section*{CONFLICT OF INTERESTS}

The authors declare no conflict of interest on the topic covered.

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