et al. and the other studies [1, 2] looked at cross-sectional data or results directly preceding dialysis.

Ellam and his colleagues have to be congratulated for a thorough effort highlighting gender differences in nutritional burden by level of eGFR. Their study raises a number of important questions that should be investigated further, such as longitudinal changes in nutritional needs by gender and the important role of gender and societal context on how renal nutritional advice is being implemented.

CONFLICT OF INTEREST STATEMENT

None declared.


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Soluble Flt-1 release response to heparin use: implications for dialysis patients?

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Soluble Flt-1 is the soluble form of the vascular endothelial growth factor (VEGF)-receptor 1 (sVEGF-R1), which results from an alternative splicing of the VEGF-R1 transcript, as well as from the cleavage and ectodomain shedding of membrane-bound VEGF-R1 [1, 2]. The primary (known) function of sFlt-1 is to antagonize VEGF and placental growth factor (PIGF), acting as a trap for the agonist and for signalling receptor components [1]. Since VEGF, a protein with a high specificity for endothelial cells, is important, even crucial, for both angiogenesis (the growth of new blood vessels) and the maintenance of endothelial cell health and microvasculature
in the basal state, sFlt-1 has been recognized as a natural anti-angiogenic and anti-endothelial factor [1, 3].

Despite being a secreted, soluble protein lacking a transmembrane domain, sFlt-1 can be stored within the tissue or in the vicinity of the cells that produce it by binding to heparan sulphate on the cell surface and/or in the extracellular matrix through its heparin-binding domain [4]. Local retention of sFlt-1 may be protective against excess VEGF, for example, in maintaining the vascularity of normal corneas [5].

In contrast, the release of sFlt-1 into the circulation, likely reflecting a failure of local retention, could affect remote organs by indiscriminate neutralization of VEGF [4]. In pre-eclampsia, for example, secretion of high amounts of sFlt-1 by the placenta, in addition to creating an anti-angiogenic environment predisposing patients to generalized endothelial dysfunction and cardiovascular disease (CVD), is responsible for the classic renal lesions associated with this condition, namely proteinuria and glomerular endotheliosis [6].

Endothelial dysfunction and impaired angiogenesis are also common in chronic kidney disease (CKD)/end-stage renal disease (ESRD), with both conditions being related to adverse clinical outcomes, such as increased risk of CVD and high mortality [7, 8]. Di Marco et al. [9] showed that moderately, albeit chronically, increased sFlt-1 levels [median plasma levels in CKD5 of 135 (interquartile ranges 100–305) pg/mL] correlate with markers of endothelial dysfunction and the incidence of cardiovascular events in these patients. Moreover, increased sFlt-1 levels (>142 pg/mL) in patients undergoing haemodialysis (HD) were shown to be an independent risk factor for cardiac and all-cause mortality [10, 11].

The mechanisms underlying sFlt-1 production, storage and release in CKD are unclear, but circulating sFlt-1 concentration appears to be independently and inversely associated with estimated glomerular filtration rate [9, 12–14].

In this issue of Nephrology Dialysis Transplantation, Lavainne et al. showed that heparin use, rather than renal function, is the factor that most determines circulating sFlt-1 concentrations in patients with ESRD. Heparin use was shown to be responsible for a dramatic (average, 25-fold) increase in serum sFlt-1 levels during dialysis. Dialysis sessions were shown to be accompanied by a marked increase in sFlt-1 levels, peaking at 15 min and returning to baseline at 4 h, with a mean peak concentration during dialysis of 2551 pg/mL. They found that this increase was strongly associated with heparin use, but was independent of dialysis modality. When heparinization procedures were identical, there were no differences in sFlt-1 levels between patients undergoing high-flux HD and those undergoing haemodiafiltration (HDF). When unfractionated heparin (UH) and low-molecular-weight heparin (LMWH) were omitted during dialysis, sFlt-1 levels did not increase significantly. As UH is currently used only for HD, with LMWH being used as maintenance anticoagulation for HD and HDF, peak serum sFlt-1 levels were significantly lower during HDF than during HD sessions.

The previous studies show that heparin strongly induces sFlt-1 release by displacing heparan sulphate-bound sFlt-1 from cells/extracellular matrix in humans as well as in experimental models [4, 14, 15]. The findings of Lavainne et al., however, emphasize that patients undergoing HD, in whom angiogenesis and microvasculature are already compromised, are regularly (thrice weekly for several months) exposed to very high sFlt-1 levels, levels observed normally only in women with pre-eclampsia.

A puzzling point of their study, however, is related to the source of sFlt-1 released during dialysis. They showed that monocytes do not respond to heparin stimulation in vitro, but that serum drawn 1 h after the start of the HD session, but not pre-dialysis serum, induced a late release (16 h) by human monocytes of sFlt-1 by stimulating the synthesis of sFlt-1 messenger RNA. These findings confirm that monocytes are an important site of sFlt-1 production in patients with CKD/ESRD, probably contributing to the low-grade chronic increase in sFlt-1 levels in these patients [9]; but do not explain the sharp increase during the first minutes of an HD session. As heparin is a well-known inducer of sFlt-1 release from endothelial cells, smooth muscle cells and placenta [4, 14, 15], it is likely that endothelial cells/arterial walls are the major sources of sFlt-1 released during dialysis.

But what then is the significance of increased circulating sFlt-1 in these patients? In the long term, elevated sFlt-1 levels may contribute to the impairment of endothelial repair, chronically compromising endothelial function and the microvasculature [6, 9]. As VEGF also possesses direct vasoactive properties, however, excess sFlt-1 may inhibit VEGF-induced vasodilatation in the short term, causing acute haemodynamic changes [6, 16]. The disruption of endothelial homeostasis by high circulating sFlt-1 levels may lead to systemic effects, including widespread endothelial damage. However, it may have a more adverse impact at local sites of paracrine and autocrine loops, where constitutive VEGF signalling and bioavailability are needed to maintain endothelial fenestrations (e.g. in the kidneys) and/or vessel and oxygen homeostasis (e.g. in the heart) [6, 17–20]. sFlt-1 may interfere with or worsen kidney function in these patients, similar to its effects in pre-eclampsia. Recently, however, much attention has been paid to the association between elevated circulating sFlt-1 and a wide number of heart diseases and cardiovascular conditions, suggesting that sFlt-1 concentration may be a marker of these conditions [12, 13, 19, 21].

Patients undergoing HD have markedly higher cardiac mortality rates than the general population, suggesting that repetitive HD-induced myocardial ischaemia may contribute to the high-cardiac event rate in these patients [22–24]. Increased sFlt-1 concentrations may contribute to the intra-dialytic reduction in myocardial blood flow occurring within the first 30 min of the start of HD, and related regional myocardial ischaemia by causing, together with other acute dialysis-related factors, vasoconstriction/haemodynamic instability. Although this point warrants further exploration, it would be a reasonable implication of the peak elevations of sFlt-1 associated with endothelial and heart dysfunction during dialysis. However, the chronic adverse effects of elevated sFlt-1 on capillary density in the hearts of ureaemic patients, thus rendering them more sensitive to the development of cardiac ischaemia, are supported by experimental studies [18, 19]. Systemic elevations in sFlt-1 significantly decreased vascular...
density even in control, healthy animals, suggesting that sFlt-1 alone, even in the absence of comorbidities, may cause cardiovascular complications [19]. Vascular risk factors other than sFlt-1 are present in patients and rats with CKD/ESRD, however, resulting in pathological states such as endothelial dysfunction, anti-angiogenesis and inflammation [25]. Elevated sFlt-1 concentrations in the setting of renal disease would render endothelial cells much more sensitive to these factors, or vice versa, thus worsening the progression and/or severity of the disease [26, 27].

However, a cardioprotective or anti-atherosclerotic activity of sFlt-1 cannot be excluded [14, 28]. Matsui et al. [14] recently showed that heparin loading induces sFlt-1 release in control, healthy subjects and in CKD patients, but that the final, post-heparin sFlt-1 concentrations levels are lower in patients. They hypothesized that, at baseline conditions (pre-heparin), patients have elevated circulating sFlt-1 levels, but reduced amounts of stored sFlt-1 (local retention). As post-heparin sFlt-1 levels represent the total amount of sFlt-1, their results suggest that the production of sFlt-1 is lower in CKD patients than in control subjects. This reduction in sFlt-1 would reflect an impaired capacity to antagonize excess VEGF/PIGF, creating a pro-atherosclerotic milieu in patients with CKD [29] and suggesting a protective effect for locally retained sFlt-1. Further studies are needed to determine the factors governing retention versus release of sFlt-1 in patients with decreased renal function.

The clinical consequences of increased circulating sFlt-1 during dialysis remain unclear. Since, however, the outcomes of dialysis patients remain unsatisfactory, any effort to improve their prognosis and our understanding of dialysis-induced cardiac injury would be welcome. Lavainne et al.’s study offers a number of ideas for further research and clinical evaluation, from the use of circulating sFlt-1 concentration as an additional parameter for the assessment and establishment of optimal dialysis procedures to the investigation of its possible causal role in the pathogenesis of CVD in ESRD patients.

Soluble Flt-1 (molecular weight of 85–120 kDa) cannot be filtered out by any dialysis membrane. However, due to its electrostatic surface potential, sFlt-1 can be adsorbed by apheresis columns, with dextran sulphate–cellulose columns being superior to heparin-based columns in removing circulating sFlt-1 in women with pre-eclampsia (82 versus 13% reduction after the first run) [30]. Because heparin is the main trigger of sFlt-1 release during dialysis, minimization of heparin use would be the optimal choice for avoiding the undesirable effects of sFlt-1 in these patients. The use of alternatives to heparins (e.g. citrate anticoagulation, citrate dialysate and heparin-coated membranes) or heparin-sparing dialysis modalities (predilutional heparin-free HDF versus conventional HD) may be appropriate. To our knowledge, other therapeutic options targeting circulating sFlt-1, including administration of recombinant ligands (e.g. VEGF) or sFlt-1 neutralizing antibodies, are not available for human use.

In summary, the paper by Lavainne et al. showed that heparin induces sFlt-1 release in patients undergoing heparin-based dialysis (especially those undergoing HD). sFlt-1 is bound to heparan sulphate on the cell surface/extracellular matrix, being released by heparin because of competitive binding at its heparin-binding site. Although endothelial cells/vessel walls are strong candidates, the primary source of sFlt-1 still remains to be determined. Regardless of its source, the concentrations of sFlt-1 in patients undergoing heparin-based dialysis may be high enough to contribute to the exacerbation of widespread endothelial dysfunction, thus compromising the endothelial/microvasculature homeostasis in organs like the kidneys and heart.

**Figure 1:** Schematic representation of sFlt-1 release in response to heparin use and possible implications for dialysis patients. Heparin use during dialysis sessions increases circulating sFlt-1 by displacing heparan sulphate-bound sFlt-1 from cells/tissues that produce it (e.g. endothelial cells/vessel walls). Release of sFlt-1 into the circulation can affect remote organs by indiscriminate neutralization of VEGF. In dialysis patients, in whom angiogenesis and microvasculature are already compromised, high sFlt-1 levels may contribute to the exacerbation of widespread endothelial dysfunction, thus compromising the endothelial/microvasculature homeostasis in organs like the kidneys and heart. While many factors are involved in the complex pathogenesis of cardiac injury in ESRD, sFlt-1 may be important in the development of dialysis-associated CVD.
however, elevated circulating sFlt-1 could worsen widespread endothelial dysfunction in patients with ESRD, as well as affecting remote target organs, such as the heart (Figure 1). Due to the repetitive nature of this insult (thrice weekly for several years), sFlt-1 may be important in the development of dialysis-associated CVD.

**CONFLICT OF INTEREST STATEMENT**

None declared.

(See related article by Lavainne et al. Heparin use during dialysis sessions induces an increase in the antiangiogenic factor soluble Flt1. *Nephrol Dial Transplant* 2014; 29: 1225–1231.)

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