Hemodialysis vascular access failure: novel pathophysiological mechanisms and therapeutic strategies

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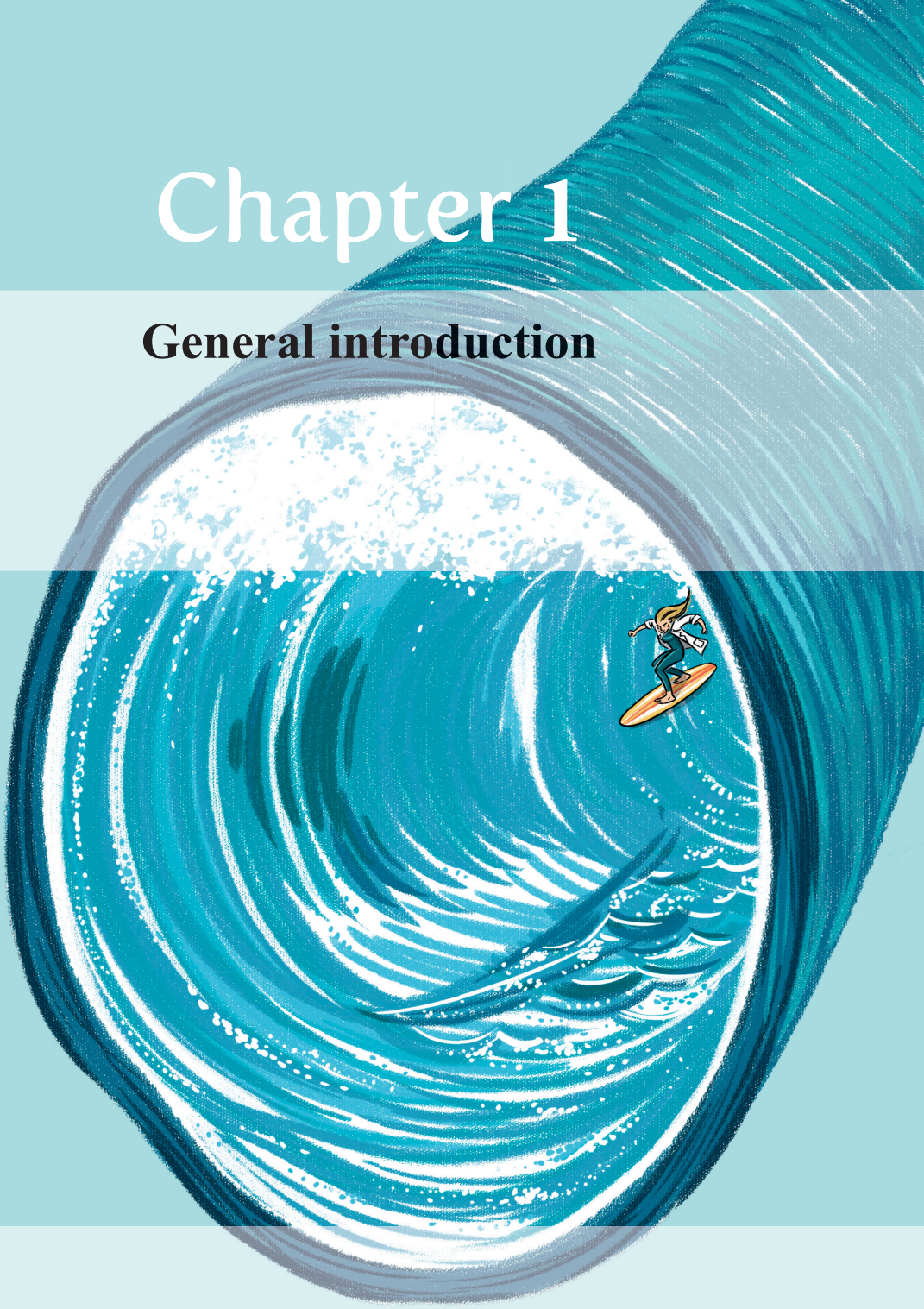
Chapter 1

General introduction



What is end stage renal disease?

The kidneys are complex organs traditionally known for their role in excretion of waste and excess water from the body. Besides, they also perform a spectrum of other functions essential for health such as helping to maintain blood pressure and assuring bone integrity. As they carry out these functions the kidneys work cooperatively and interactively with other organs systems, particularly the cardiovascular system.

Kidney disease can ultimately lead to the loss of kidney function and it can develop rapidly—acute kidney injury (AKI) or have long term pathology—chronic kidney disease (CKD).

Diabetic nephropathy followed by hypertension are the most common causes of CKD. When not treated, CKD can progress to end-stage renal disease (ESRD). ESRD is a terminal illness defined as having a glomerular filtration rate less than 15 mL/min. The development of CKD and its progression to ESRD remains a major source of reduced quality of life and significant premature mortality. Over the last decades the number of ESRD patients steadily increased worldwide. In the Netherlands, on January 2017, there were 17.132 ESRD patients and each year there are about 2000 new reported cases of ESRD[1].

ESRD is fatal without renal replacement therapy (RRT).

How kidney failure treated and what is renal replacement therapy?

Kidney failure may be treated with hemodialysis (HD), peritoneal dialysis (PD) or kidney transplantation. On January 2017, in the Netherlands 32% (5450) patients were on HD and 63% (10812) received kidney transplant[1].

In hemodialysis treatment, extracorporeal removal of waste products and extraction of fluids is carried out directly via the blood, whereas peritoneal dialysis is carried out via the peritoneal cavity.

Treatment with HD may be performed at a dialysis unit or at home. In-center HD treatments are usually performed three times a week. Home HD and PD is generally done daily at home.

According to the 2017 United States Annual Renal Data Report[2] in 82% of the countries, HD is still the most common way of renal replacement therapy.

What is hemodialysis vascular access?

To be able to start hemodialysis therapy, a proper access site to a blood vessel with a high blood flow is required.

When urgent or emergent HD is needed central venous catheter (CVC) represent the only means for vascular access option (Figure 1a). However, this direct form of vascular access is not preferred for chronic dialysis treatment due to high risk of infections and central venous stenosis[3,4].

To create a vascular access point in the upper extremity is preferred approach. Anatomical superficial location of veins in the low arm makes percutaneous cannulation easier and more suitable for regular use.

However, the blood flow in the low arm veins is not high enough for efficient dialysis. To allow high blood flow so that large amount of blood can pass through the dialyzer, a surgical connection between high pressure arterial system with a low pressure venous system is needed. A direct connection between the native artery and vein is called an arteriovenous fistula (AVF) (Figure 1b), when a prosthetic interposition between an artery and a vein is used it is called an arteriovenous graft (AVG) (Figure 1c).

According to the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI)[5], the placement of AVFs is currently regarded as the best available option for permanent vascular access, especially because of the lower rate of mortality, infections, thrombotic complications and a higher patency when compared to AVGs or CVCs[6-8]. However, a downside of the AVF is that it needs to be planned at least one or two months before starting HD, a time required for the proper "maturation" of the AVF.

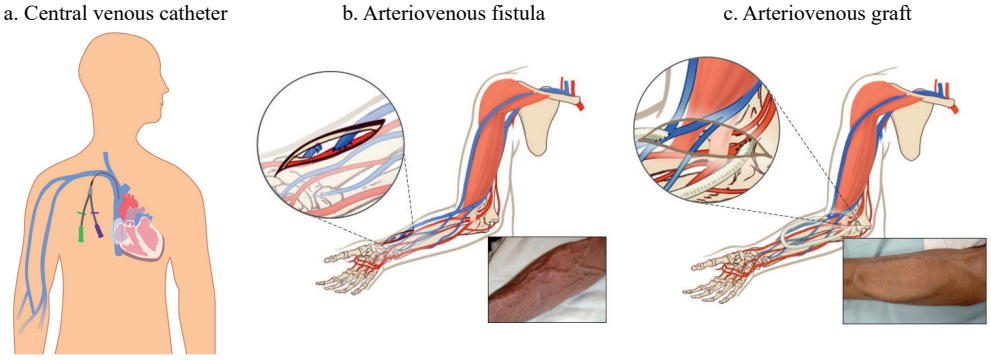


Figure 1. Types of hemodialysis vascular access.
adapted from Atlas of Dialysis Vascular Access, Tushar J. Vachharajani

What is arteriovenous fistula maturation?

According to the NKF KDOQI guidelines an AVF is matured and functional when the fistula has the flow of > 600 mL/min, the vein has a minimum diameter of 6 mm[2] and does not exceed the depth of 6 mm, and the margins are clearly identifiable.

For an AVF to successfully mature several functional and structural adaptations to the inflow artery and outflow vein are required. The connection of a low-pressure vein to the high-pressure arterial circulation results in an immediate increase of blood flow and wall shear stress (WSS) through both the inflow artery and the outflow vein[9-11].

On a biological level, changes in WSS and wall tension are sensed by vascular endothelial cells, that convert hemodynamic stimuli into biochemical signals such as production of nitric oxide (NO) and growth factors that can trigger relaxation of vascular smooth muscle cells (VSMCs) resulting in acute vasodilatation[12-14].

Upregulation of matrix metalloproteinases (MMPs) results in matrix degradation and restructuring of the vascular scaffold leading to luminal expansion[15] but also to thickening especially of the venous wall[16,17]. Finally, successful vascular remodeling restores WSS toward normal levels in a vessel with increased blood flow and helps maintain luminal diameter[18-20], a key hallmark of successful AVF remodeling.

Current concept of AVF maturation postulates that both, degrees of intimal hyperplasia (IH), which thickens the venous wall, narrows the luminal area, and predisposes to intravascular thrombosis together with adaptive restructuring of the vascular wall (outward remodeling [OR]), leading to luminal expansion of the arteriovenous conduct will ultimately determine luminal dimensions, fistula flow and patency[21,22] (Figure 2).

What does it mean an arteriovenous fistula maturation failure?

While AVFs remain the most common vascular access, complications related to vascular access are one of the most common causes of hospitalization and morbidity in hemodialysis patients with approximately of $1 billion spent annually on health care costs[23].

The utility of AVFs is hampered by two distinct causes of failure: (1) initial failure to mature, and (2) dysfunction of mature AVFs due to stenotic lesions in the venous outflow tract.

Recent quantitative study of the outcomes of fistula patency and maturation reports that the primary and secondary patency rates for fistulas at one year is an average 64%, and 79% respectively. For fistulas that were reported as mature, mean time to maturation was 3.5 months, however only 26% of created fistulas were reported as mature at 6 months and 21% of fistulas were abandoned without use[24].

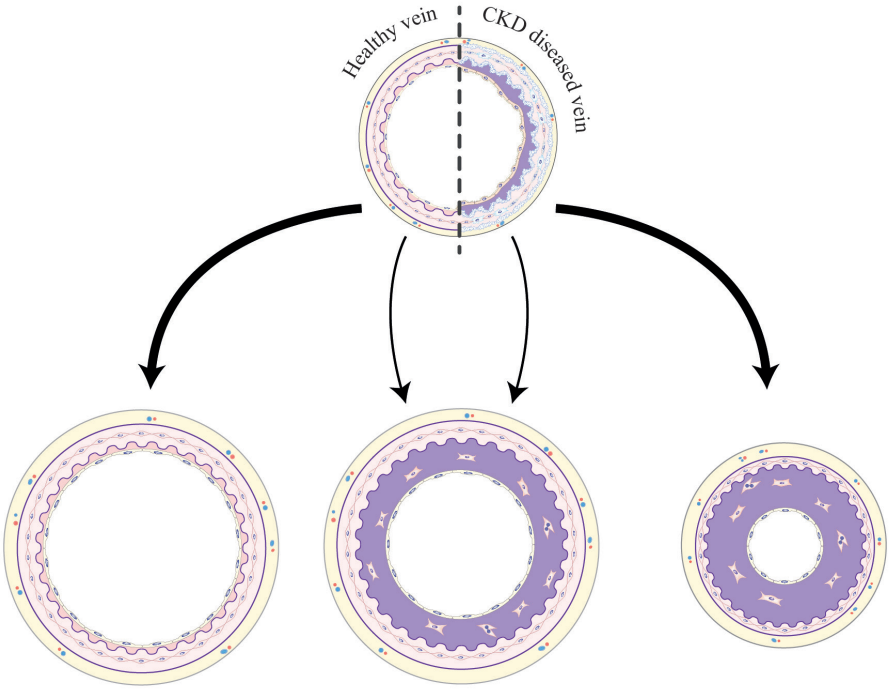


Figure 2. Vascular remodelling after fistula creation.
Healthy vein has the potential for successful outward remodelling (top left), whereas adequate maturation may be partially hindered by CKD-induced pre-existing intimal hyperplasia (IH) (top right). The net resultant of IH and outward remodelling may determine ultimate luminal calibre. adapted from Rothuizen *et al.*, Nephrol Dial Transplant. 2013 May;28(5):1085-92

The exact mechanisms that lead to AVF maturation failure remain unknown, but both impaired OR and formation of IH are regarded as primary contributors to this pathophysiology[21].

As previously discussed, the creation of an AVF results in an increase in shear stress, which causes vascular dilation in an attempt to return shear stress levels back to normal. As we try to create AVF in patients with severe vascular disease, it is possible that this conventional dogma may not always hold true. As an example, vascular calcification seen in elderly patients with diabetes mellitus, prevents endothelium to secrete mediators that are required for flow-mediated vasodilation.

Geometrical configuration of AVF may cause creation of difference in shear stress levels across the venous segment. In particular, at the site of arteriovenous anastomosis there are regions of low shear stress prone to inflammation. Increase in inflammation activates deposition of extracellular matrix (ECM) proteins, production of cytokines and growth factors, activation of proliferation and migration of VSMCs—all together creating focal areas of intimal hyperplasia and vasoconstriction[25-27]. There is an ongoing debate

about the anatomical origin of cells that are responsible for venous stenotic lesions in arteriovenous fistulas[28-32]. This issue will be further elaborated in **chapter 5** of this thesis.

The fact that AVF being the most favored access, exhibits failure rates that are among the highest for any elective surgical procedure, underscores the enormity of the issue of hemodialysis access dysfunction.
The focus of this thesis will be primarily on the novel strategies to prevent arteriovenous fistula maturation failure.

Are there other alternatives to vascular access besides an arteriovenous fistula?

As mentioned before, patients with ESRD require surgery to create a vascular access site for hemodialysis. For this purpose, native veins are generally preferred due to superior patency rates when compared to prosthetic grafts, but often unavailable due to preexisting vascular pathology often seen in patients with CKD[33,34]. The failure of synthetic vascular grafts predominantly results from the development of intimal hyperplasia ultimately leading to graft occlusion, and a relatively high risk of infectious complications[3,35,36].

In recent years, tissue engineering strategies have made substantial progress to improve vascular access. Tissue engineered blood vessels could offer a better alternative to synthetic AV-grafts, when it is not possible to create an AV-fistula. Indeed, tissue engineered blood vessels (TEBVs) can be tailor-made, are devoid of pre-existing vascular diseases and have the potential to adapt to changing hemodynamic conditions.

Can we engineer vascular tissue?

Several strategies to develop tissue engineered grafts have been described[37]. The majority of these approaches include complex *in vitro* preparation steps, decellularized scaffolds, or the incorporation of synthetic materials to generate TEBV. Ideally, vascular replacement should comprise of completely autologous cellular tissue, to avoid host-immune reaction and hold the ability to remodel *in vivo*.

In our research group we developed a novel method to generate TEBVs by utilizing the foreign body response directed towards subcutaneously implanted polymeric rod that culminates in the formation of a fibrocellular tissue capsule (TC). Upon extraction of the polymer rod several weeks after implantation, the remaining tissue capsule is grafted into the vasculature, whereupon it differentiates into a blood vessel[38].

Thus far, the origin of cells in these TCs remained unknown. In **chapter 6** of this thesis, this topic will be discussed further.

Scope of this thesis

Previously, we established a unique murine model of arteriovenous fistula, which has a configuration similar to the one most frequently used in humans (venous end-to-arterial side)[25]. This thesis includes subsequent studies of this model designed to evaluate new therapeutic strategies aimed to improve patency of AV-fistulas and to unravel the pathophysiology of AVF failure.

In addition, we further expand our understanding on the cellular origin of tissue capsules for autologous tissue engineering, as an alternative to AV-grafts.

It is known that the process of vascular adaptation after AVF creation is associated with an excessive inflammatory response characterized by the infiltration of macrophages and lymphocytes as well as the up regulation of pro-inflammatory cytokines[30,39].

In chapter 2 and 3 of this thesis we further examined the contribution of inflammation to AVF failure.

In **chapter 2** we studied the complex role of toll-like receptor 4 (TLR4) homologue -RP105 in AVF remodeling. Here we hypothesized that in mice deficient for RP105 pro-inflammatory TLR4 signaling is upregulated, which results in worsening of AVF maturation.

We created AVFs in RP105 deficient mice to study the effect on AVF maturation *in vivo*. In addition, in series of *in vitro* studies we defined cell-specific effects of RP105 on macrophages and VSMCs.

In another study, described in **chapter 3**, we evaluated the feasibility and efficacy of prednisolone—a potent anti-inflammatory drug. To improve local biological activity and reduce systemic side-effects prednisolone was encapsulated in liposomes, a potent vehicle for targeted drug delivery to inflamed organs.

In **chapter 4** we assessed the role of the relaxin (RLN2) pathway in AVF remodeling. Relaxin is a hormone exhibiting its action on the vasculature via interaction with its receptor (RXFP1), resulting in vasodilatation, ECM remodeling and decreased inflammation—favorable effects for successful AVF maturation. In view of the emerging role of the RLN2-RXFP1 axis in vascular remodeling and inflammation, we examined the consequences of disturbing this hormone-receptor balance in AVF maturation. For this purpose, we used murine AVF model in which we studied the effect of RXFP1 deficiency on fistula remodeling. Furthermore, we determined the effects of RXFP1 deficiency on the phenotype and function of VSMCs *in vitro*.

The pathophysiology of arteriovenous fistula maturation failure is associated with impaired outward remodeling and intimal hyperplasia[21]. In **chapter 5**, based on the work of Liang and co-workers[29] the on-going debate about the cellular origin of cells forming intimal hyperplasia and venous stenosis in AVFs is discussed.

In parallel with testing new therapeutics to improve maturation and patency of native AVFs, our group is working on developing TEBVs, which could offer a suitable alternative for arteriovenous conduits, circumventing the limitations of synthetic grafts and avoiding the need for maturation of fistulas.

In **chapter 6**, a lineage tracing study is described to elucidate the contribution of bone marrow derived cells to TEBV formation. We established a rat model where cells of the hematopoietic lineage are labeled with green fluorescent protein. For the clinical application of the *in situ* engineered vascular grafts, we focus on patients with ESRD that require a vascular access for hemodialysis. Therefore, we combined bone marrow lineage tracing with a model of CKD to investigate the effect of chronic kidney failure on tissue capsule composition.

Finally, **chapter 7** gives an overall summary of the research presented in this thesis and discusses future prospective on vascular access for hemodialysis to reduce morbidity in ESRD patients.

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