Unraveling mechanisms of vascular remodeling in arteriovenous fistulas for hemodialysis

Wong, C.Y.

Chapter 8

Summary and Discussion

Thanks to the invention of the first dialysis machine by Kolff in 1943 combined with the development of vascular access methods that was started by Scribner[1, 2], there finally was a treatment for end stage renal disease (ESRD) patients. At present, kidney transplantation has become the preferred modality in kidney replacement therapy. Although the number of patients with a functioning donor kidney in the Netherlands is increasing, the shortage of suitable donors still persists. The remaining group of patients that is dependent on dialysis is therefore still a considerable one (40%)[3]. Hemodialysis is the most widely used option in chronic dialysis treatment and requires a suitable vascular access. Unfortunately, this vascular access suffers from a high number of failures responsible for a high morbidity and health care costs.

In **chapter 2** the pathophysiology of arteriovenous fistula (AVF) failure is discussed. Although the exact pathophysiology of AVF failure remains unclear, there is a general consensus among researchers that it is multifactorial. At an anatomical level, the two main processes that are responsible for AVF failure are intimal hyperplasia (IH) and inadequate outward remodelling (OR). The vascular adaptation process is mainly driven by altered haemodynamical factors that starts with the surgical procedure in which the low-pressure vein is connected to the high-pressure arterial system. The result is an immediate increase in blood flow through both the feeding artery and the draining vein[4, 5]. This rapid increase in flow results both in passive vascular distension and in an nitric oxide (NO) driven active distension in which the endothelial cells(ECs) produce NO that in its turn relaxes the vascular smooth muscle cells (VSMCs)[6, 7]. Concomitantly, a more structural form of vascular remodelling is initiated as the body is trying to normalize the increased wall shear stress and wall tension by increasing the luminal diameter and increasing vessel wall thickness, respectively[8]. It is believed that the endothelial and vascular smooth cell are the key players, and that the process starts with the EC that converts haemodynamic stimuli into biochemical signals such as vasodilating agents, growth factors, cellular adhesion molecules, cathepsins and matrix metalloproteinases (MMPs). Intimal hyperplasia is the pathologic lesion in AVFs that may result in stenosis and ultimately thrombosis. At a histological level, IH is characterized primarily by alpha smooth muscle actin (α-SMA) positive cells from which the vast majority exhibit a myofibroblast or synthetic VSMC phenotype. The pathophysiology of intimal hyperplasia is believed to be multifactorial and has been well described in literature. One of the factors associated with IH is unfavourable haemodynamic profile in the form of low flow and oscillating flow pattern. Also, ESRD associated pathologies such as a chronic inflammatory state[9, 10], uremia[11, 12] and increased baseline levels of oxidative stress[13] all seem to contribute to IH formation. Moreover, pre-existing venous IH and vascular calcification both can influence AVF maturation in a negative way. The net resultant of the unbeneficial IH formation and beneficial OR will

ultimately determine luminal calibre, flow and long term patency. Due to the potential positive contribution to AVF maturation and its assumed role in luminal calibre preservation, we pledge for more research emphasis on the role of outward remodelling.

In order to study the complex effects that is involved in AVF failure, we decided to utilize an in-vivo animal model that mimics the human situation as closely as possible. Numerous animal models have been developed over het past decades in order to unravel vascular access related pathology. Large animals such as baboons, pigs, goats, sheep and dogs have the advantage that the vascular dimensions approaches the human situation more closely than in smaller animals. This is important due to the fact that hemodynamical factors are considered to be an important contributor to AVF failure. These larger animal models are very suitable for experimental set-ups aimed at surgical and interventional techniques. However, the high costs and moderate availability associated with large animals have to be taken into account. In contrary, smaller animals such as rodents are less costly and are more easily available. More importantly, due to the availability of transgenic mice, murine models are very well suited for studies aimed at unravelling the molecular mechanisms. However, due to the small dimensions a surgeon that is skilled in microsurgery is a requirement. In 2006, Castier et al.[14] introduced an improved murine model of AVF failure in which he was the first to describe a vascular anastomosis using only sutures, thus eliminating the presence of an intravascular catheter. However, the arterial end to venous side vascular anastomosis configuration that was used, does not resemble the most frequently used configuration that is used in humans. This has a great impact on the hemodynamical profile in the AVF.

In **chapter 3** we describe a new murine model that we have developed together with the group of Roy-Chaudhury in which we incorporate a venous end to arterial side anastomosis, resembling the human situation as closely as possible. In this model we connected the right common carotid artery to an ipsilateral branch of the external jugular vein. We analysed the AVF histologically at different time points (day 0, 7, 14 and 28) and observed that IH is mainly located in the venous outflow tract. Chronologically, these lesions appear to start with a mural thrombus that in time is popularized by alpha smooth muscle actin (α-SMA) positive cells. Both the location and the cellular composition of the IH resemble the human vascular pathology that is seen in failed AVFs. The growth of IH increases in a linear fashion in the course of time. The second process that will determine the luminal area is outward remodeling. In our model, OR is only present during the first two weeks after AVF creation. Due to the progressive growth of IH during the whole period and the ceasing of OR after 2 weeks, the luminal area will decrease starting from week 2 ultimately leading to an occlusion of the AVFs at week 4. Postoperatively, we observed a disrupted lining of endothelial cells that that seemed to grew back in a time dependant manner.

Following AVF creation, CD45 positive cells seem to migrate into the vessel wall from the neovascularization in the adventitia. This is different from the traditional concept that the recruitment of inflammatory cells starts from the luminal side. The consequence of this finding is that future therapies directed against the influx of (inflammatory) cells should perhaps be targeted against the adventitia. Moreover, this finding confirms the involvement of inflammation in AVF failure.

The assessment of the patency of the murine AVF using only visual and physical confirmation can sometimes be difficult. Other visualisation techniques such as ultrasonography and magnetic resonance imaging can aid in this assessment. Unfortunately, we were not able to successfully assess the patency of the AVF using the abovementioned techniques due to technical reasons. As an alternative, we used Near Infrared Fluoroscopy (NIRF) This method requires an intravenous injection of indocyanine green, which is then visualized using a specialized camera that captures video images in real-time. This technique proved to be a safe and technical easy to perform vascular patency assessment in our model. In order to help in the surgical training, we described (**chapter 4**) the technically challenging procedure in detail in a step-by-step protocol that also includes a video that demonstrates the key steps.

As described above, following AVF creation the involved vessels need to adapt (mature) in order to become an adequate vascular access point for haemodialysis. The complex process requires the vessel wall to remodel. The vascular wall consists of multiple structures, from which the extracellular matrix (ECM) takes up most of the volume. One of the most prominent proteins in the ECM is elastin. Elastin production begins with the synthesis and secretion of the soluble precursor tropoelastin by VSMCs and is then subsequently crosslinked by lysyl-oxidase to form elastic fibers. These fibers are located concentrically in a group-wise manner, forming the elastic laminae. This structure is responsible for the mechanical properties that is unique and essential for proper function.

In **chapter 5** we studied the effect of elastin on vascular remodelling in our murine AVF model by incorporating a mouse that is haplodeficient for the elastin gene ($eln^{+/-}$). In literature the phenotype has been well described, although it is mainly focused on the artery. Histologically, we observed a reduced amount of elastic fibers in the vein of $eln^{+/-}$ mice prior to surgery, without a compensatory increase in the number of rings of elastic lamellae as is seen in the aorta of these animals[15]. After AVF creation, the amount of elastic fibers in the venous vessel wall is reduced in both the $eln^{+/-}$ and wild type (WT) mice. At 3 weeks the mRNA expression levels of mELN was 53% lower when compared to WT mice. Interestingly, with this decrease in elastin presence we observed an increased OR (21%) in $eln^{+/-}$ mice. We hypothesize that this is due to the reduced mechanical strength in blood vessels of $eln^{+/-}$ mice. This mechanical effect is elegantly demonstrated in a study by Li et al.[15], in which the aorta of $eln^{+/-}$ mice show a reduced extensibility at higher pressures.

Interestingly, elastin did not seem to have an effect on IH formation, though it has been shown that elastin has an anti-proliferative effect on VSMCs. It is not clear why this effect was not seen in our AVF model. Perhaps this is due to the fact that in our model IH is initiated and dependant on the primary thrombus formation after vascular injury, in contrast to the pathophysiology that is seen in haplodeficient mice and humans[15].

While the size of the intimal area did not differ between the WT en $eln^{+\backslash -}$ mice, we did observe a larger content of α-sma positive cells in the venous outflow tract of the $eln^{+/-}$ mice. The latter results from a higher density of α-sma positive cells in the intima. In addition, the enhanced OR response in $eln^{+/-}$ mice also resulted in a larger VSMCs content in the adventitia and media. This observation supports the hypothesis that VSMC proliferation in the initial phase after AVF surgery is required in order to keep pace with the expansion of the venous outflow tract, as demonstrated previously in flow induced arterial OR in rats[16].

The data from this study in which we observed an enlarged OR in the elastin haplodeficient mice provides support for the hypothesis that low elastin content in the venous outflow tract might facilitate AVF maturation. Therapies aimed at lowering the elastin content, such as topical application of pancreatic elastase on AVFs[17], would therefore be a viable strategy to improve AVF maturation.

Animal studies have shown that the adaptive response that occurs upon AVF creation, is characterized by marked vascular inflammation as illustrated by the infiltration of macrophages and lymphocytes[18] as well as the up regulation of pro-inflammatory cytokines[19]. In addition, clinical studies suggest an association between increased inflammatory blood markers and AVF dysfunction[9, 20]. In line with these clinical findings, we observed a marked influx of CD45-positiveinflammatory cells in the vessel wall after AVF creation in our murine model (**chapter 3**), confirming the involvement of inflammation in AVF failure.

Glucocorticoids (GCs) are powerful anti-inflammatory drugs that act through binding to cytosolic glucocorticoid receptors in target cells, leading to a reduction in the expression of pro-inflammatory cytokines and diminished recruitment of inflammatory cells[21, 22]. Despite these potent anti-inflammatory effects, chronic and systemic therapeutic use of GCs is limited by the high incidence of serious adverse effects[23]. Moreover, as a result of rapid clearance from the circulation, systemic administration of GCs results in low efficacy of drug delivery at the target location. Therefore, encapsulation of GCs in liposomal nanoparticles holds great potential, as it can hold their payload while circulating in het vasculature until they extravasate and accumulate at sites of inflammation as a result of enhanced vascular permeability in inflamed tissues, thereby reducing possible systemic side effects and more importantly, increasing the local availability of its payload.

In **chapter 6** we set out to elucidate the role of inflammation in AVF failure, by suppressing the inflammatory reaction in our murine model using liposomal prednisolone (L-Pred). Firstly, we have demonstrated by using near infrared fluoroscopy (NIRF) and immunohistochemistry that the liposomes extravasate and accumulate in macrophages in the anastomotic area of the AVF. More specifically, we believe that the liposomes extravasate from the neovascularization in the adventitia. Interestingly, this is the same area in which IH is most prominent. Therefore, delivery of GCs in this area would be desirable.

At a morphometrical level, treatment with L-Pred resulted in an increase in OR of 27%, a 47% increase in luminal area and a trend towards a higher IH area when compared to the PBS treated group. Control groups that were administered either liposomal PBS or unencapsulated prednisolone did not show any effect on the morphometry. This interesting finding is accompanied by the anti-inflammatory effect of prednisolone. We have confirmed this reaction histologically and in in-vitro experiments. Histologically, we observed an 83% reduction in the amount of CD45 positive cells in animals treated with L-Pred when compared to PBS treated controls. Looking more closely at the specific type of inflammatory cells, we observed a reduction of 86% and 51% in T-cells and granulocytes, respectively.

Interestingly, liposomes themselves contribute to the reduction of the pro-inflammatory profile of macrophages, as illustrated by the reduction of pro-inflammatory cytokines in M1 macrophages upon stimulation with L-PBS. This shift towards an anti-inflammatory profile of macrophages might have inhibited the recruitment of lymphocytes and granulocytes to the injured vessels of the AVF. Alternatively, prednisolone might have had a direct effect on lymphocytes and granulocytes as GCs can easily pass the cellular membrane of macrophages in both directions.

The exact mechanism by which liposomal prednisolone resulted in increased OR in murine AVF is not clear. We speculate that matrix metalloproteinases might be involved in this response. MMPs are a group of proteolytic enzymes involved in vascular remodeling by facilitating the turnover of extracellular matrix components such as collagen and elastin[24]. The effect of MMPs on the vascular remodeling depend on the specific MMPs that are activated and the type of vascular injury[25]. Previous studies in a porcine balloon angioplasty model in peripheral arteries revealed that treatment with the MMP-inhibitor marimastatin resulted in reduced constrictive arterial remodeling in favor of expansive remodelling[26]. In addition, a recent clinical trial evaluating the efficacy of the MMP-inhibitor doxycycline to inhibit growth of abdominal aortic aneurysms resulted in an unexpected acceleration of the aneurysmal growth[27]. These data suggest that in certain vascular disease conditions, inhibition of MMPs could enhance outward remodeling. Interestingly, elegant studies by Nieves Torres and coworkers[28] suggest a similar effect of MMP inhibiton on venous outward remodeling

in AVF. Indeed, adventitial delivery of a small hairpin RNA against the MMP ADAMTS-1, resulted in reduced macrophage infiltration, decreased MMP-9 activity and enhanced outward remodeling in murine AVF. We have demonstrated that L-pred effectively increases MMP2 and MMP9 expression in cultured macrophages. Although we have not been able to quantify MMP activity *in vivo*, we speculate that the enhanced outward remodeling in murine AVF that occurred upon treatment with L-Pred is mediated by the dampened inflammatory response and decreased MMP activity in macrophages.

In contrast to its effect on outward remodeling, no effect of L-Pred on IH in the venous outflow tract was observed. These results deviate from other preclinical studies that evaluated the therapeutic effect of GCs in other vascular injury models, that have reported a strong inhibitory effect of dexamethasone on IH[29-31]. This discrepancy may result from a difference in potency between prednisolone and dexamethasone to inhibit VSMC proliferation[32]. Alternatively, it may relate to the difference in pathophysiological stimuli that contribute to IH after arterial injury, when compared to venous IH in AVF. While hemodynamic stimuli are considered to be of vital importance for IH[33, 34], the contribution of inflammation to IH in AVF might be limited, as suggested by our results. Ultimately, the net result of vascular remodelling in the AVF was an increased luminal area in the venous outflow tract, that was mainly accountable by the OR process alone.

In conclusion, liposomal prednisolone reduces the local inflammatory response and stimulates venous outward remodeling in murine AVF. Therefore, treatment with liposomal prednisolone might be valuable strategy to reduce AVF non-maturation. Based on the results of this study, a double-blind, randomized, placebo-controlled clinical trial was initiated that will evaluate the efficacy of liposomal prednisolone to enhance AVF maturation in ESRD patients.

In **chapter 7** we study the effects of the Toll-Like Receptor 4 (TLR4) inhibitor RP105 on AVF maturation. Based on the hypothesis that TLR4 mediated inflammatory responses contribute to vascular remodeling in several pathophysiological processes, we expected an increased AVF maturation in mice that are deficient of the TLR4 inhibitor RP105. Surprisingly we observed a slight but significant reduction in outward remodeling in the venous limb of the AVFs in $RP105^{-/-}$ mice. More detailed analysis of the effects of RP105 deficiency on the inflammatory response revealed an unexpected reduction in pro-inflammatory macrophages (M1) and a strong shift towards anti-inflammatory M2 macrophages. This was accompanied by a 70% reduction in CD3+ T-cells and a reduction in MMP activity. Moreover, we observed strong effects on proliferation of smooth muscle cells of both arterial and venous origin. Since the expression of RP105 in the latter group of smooth muscle cells is much higher, the inhibitory effects on proliferation of venous smooth muscle cells , the cells that most likely are the strongest contributors to outward remodeling of the arteriovenous fistulas, are the most prominent

effects observed. These results demonstrate that the concept of inhibiting the inhibitor of TLR4 in order to stimulate the TLR4 mediated inflammatory driven outward remodeling is too simple and a better understanding of the complex interaction of the various cell types involved in fistula maturation and especially the role of RP105 in these multifactorial processes is essential before any therapeutic options can be defined on this concept.

Final conclusion

Arteriovenous fistula failure is a major clinical problem that is responsible for high morbidity and healthcare cost in patients that are in need of a chronic hemodialysis vascular access. Despite all the research that has been committed, the exact pathophysiology remains unknown. One of the objectives was to develop a murine model of AVF failure that was different compared to the existing murine models in the way that it resembles the vascular configuration in humans. In the murine model that was used prior to our newly introduced model, the anastomosis was configurated in an venous-side to arterial-end manner, whereas we introduced a configuration that consisted of an arterial-side to venous-end. This difference in configuration is important in that it leads to a different hemodynamical profile, which in itself also seems to contribute to the pathophysiology of AVF failure. We successfully created a novel murine model that showed similar histological pathologies as is seen in failed human arteriovenous fistulas. This model can be used to study the pathophysiology of AVF failure and to explore possible future therapies. Interestingly, our model showed that the characteristic intimal hyperplasia that is seen in AVF failure is firstly initiated by thrombus formation, followed by the popularisation of α-smooth muscle actin positive cells. This suggests that prevention of thrombus formation would decrease intimal hyperplasia and therefore AVF failure. However, anti-coagulatory/ anti platelet therapies in the clinical setting never have shown an improvement in maturation failure on the long term[35]. Moreover, the administration of vitamin K antagonism even seems to increase intimal hyperplasia in rats[36].

Although chronic kidney disease (CKD) seems to accelerate the development of intimal hyperplasia in mice who received an AVF[37], most of the research, including ours, were conducted in an non CKD animal. For future research we would propose to combine our model with a CKD model such as described by Kang et. al[38].

Following the development of a novel AVF model, we then focused on the unravelling of the pathophysiology behind AVF failure. We first explored the role of the extra cellular matrix protein elastin and showed that a reduction in elastin increases the outward remodelling process, due to the altered mechanical composition of the blood vessel. This concept is now

being researched in a clinical trial, in which human type 1 pancreatic elastase (PRT-201) is locally applied on AVFs[17] and arteriovenous grafts[39]. The first results are promising, but it will need to be confirmed in larger studies.

Next, we focused on the role of inflammation in AVF failure. In our AVF model we observed a marked influx of CD-45 positive cells in the vessel wall, suggesting that inflammation is involved in AVF maturation. To further explore this, we reduced the inflammatory response by administering liposomal prednisolone, which led to an increased outward remodelling and luminal area of the venous outflow tract of the AVF. Suggesting that the localised delivery of prednisolone via liposomes has a beneficial effect in AVF maturation. This hypothesis will be investigated in a double-blind, randomized, placebo-controlled trial in humans that commenced in 2015.

In contrast to the study with liposomal prednisolone, we also wanted to explore the effect on AVF maturation when the inflammation was increased via the TLR4 pathway by removing the TLR4 inhibitor RP105 using KO mice. To our surprise, we observed an unexpected anti-inflammatory effect that led to an decreased outward remodeling of the AVF. This effect may relate to a shift of macrophages towards the M2 phenotype, reduction in MMP activity and a decreased proliferation rate of VSMCs. This unexpected result towards an anti-inflammatory effect, demonstrates that the TLR4 mediated pathway is more complex than anticipated and that more understanding of this process is needed before we can define a therapeutic strategy for this pathway.

Reference List

1. Quinton W, Dillard D, Scribner BH. Cannulation of blood vessels for prolonged hemodialysis. Hemodial Int 2004 Jan 1;8(1):6-9.

2. Scribner BH, Buri R, Caner JE, Hegstrom R, Burnell JM. The treatment of chronic uremia by means of intermittent hemodialysis: a preliminary report. 1960. J Am Soc Nephrol 1998 Apr;9(4):719-26.

3. Renine.nl. 2015.

4. Saucy F, Haesler E, Haller C, Deglise S, Teta D, Corpataux JM. Is intra-operative blood flow predictive for early failure of radiocephalic arteriovenous fistula? Nephrol Dial Transplant 2010 Mar;25(3):862-7.

5. Tordoir JH, Rooyens P, Dammers R, van der Sande FM, de HM, Yo TI. Prospective evaluation of failure modes in autogenous radiocephalic wrist access for haemodialysis. Nephrol Dial Transplant 2003 Feb;18(2):378-83.

6. Cooke JP, Rossitch E Jr, Andon NA, Loscalzo J, Dzau VJ. Flow activates an endothelial potassium channel to release an endogenous nitrovasodilator. J Clin Invest 1991 Nov;88(5):1663-71.

7. Tronc F, Wassef M, Esposito B, Henrion D, Glagov S, Tedgui A. Role of NO in flow-induced remodeling of the rabbit common carotid artery. Arterioscler Thromb Vasc Biol 1996 Oct;16(10):1256-62.

8. Corpataux JM, Haesler E, Silacci P, Ris HB, Hayoz D. Low-pressure environment and remodelling of the forearm vein in Brescia-Cimino haemodialysis access. Nephrol Dial Transplant 2002 Jun;17(6):1057-62.

9. Liu BC, Li L, Gao M, Wang YL, Yu JR. Microinflammation is involved in the dysfunction of arteriovenous fistula in patients with maintenance hemodialysis. Chin Med J (Engl) 2008 Nov 5;121(21):2157-61.

10. Sezer S, Ozdemir FN, Arat Z, Turan M, Haberal M. Triad of malnutrition, inflammation, and atherosclerosis in hemodialysis patients. Nephron 2002 Jul;91(3):456-62.

11. Bolton CH, Downs LG, Victory JG, Dwight JF, Tomson CR, Mackness MI, et al. Endothelial dysfunction in chronic renal failure: roles of lipoprotein oxidation and pro-inflammatory cytokines. Nephrol Dial Transplant 2001 Jun;16(6):1189-97.

12. Ghiadoni L, Cupisti A, Huang Y, Mattei P, Cardinal H, Favilla S, et al. Endothelial dysfunction and oxidative stress in chronic renal failure. J Nephrol 2004 Jul;17(4):512-9.

13. Himmelfarb J, Stenvinkel P, Ikizler TA, Hakim RM. The elephant in uremia: oxidant stress as a unifying concept of cardiovascular disease in uremia. Kidney Int 2002 Nov;62(5):1524-38.

14. Castier Y, Lehoux S, Hu Y, Foteinos G, Tedgui A, Xu Q. Characterization of neointima lesions associated with arteriovenous fistulas in a mouse model. Kidney Int 2006 Jul;70(2):315-20.

15. Li DY, Faury G, Taylor DG, Davis EC, Boyle WA, Mecham RP, et al. Novel arterial pathology in mice and humans hemizygous for elastin. J Clin Invest 1998 Nov 15;102(10):1783-7.

16. Buus CL, Pourageaud F, Fazzi GE, Janssen G, Mulvany MJ, De Mey JG. Smooth muscle cell changes during flow-related remodeling of rat mesenteric resistance arteries. Circ Res 2001 Jul 20;89(2):180-6.

17. Hye RJ, Peden EK, O'Connor TP, Browne BJ, Dixon BS, Schanzer AS, et al. Human type I pancreatic elastase treatment of arteriovenous fistulas in patients with chronic kidney disease. J Vasc Surg 2014 Aug;60(2):454-61.

18. Wang Y, Krishnamoorthy M, Banerjee R, Zhang J, Rudich S, Holland C, et al. Venous stenosis in a pig arteriovenous fistula model--anatomy, mechanisms and cellular phenotypes. Nephrol Dial Transplant 2008 Feb;23(2):525-33.

19. Nath KA, Kanakiriya SK, Grande JP, Croatt AJ, Katusic ZS. Increased venous proinflammatory gene expression and intimal hyperplasia in an aorto-caval fistula model in the rat. Am J Pathol 2003 Jun;162(6):2079-90.

20. Kaygin MA, Halici U, Aydin A, Dag O, Binici DN, Limandal HK, et al. The relationship between arteriovenous fistula success and inflammation. Ren Fail 2013 Sep;35(8):1085-8.

21. Cronstein BN, Kimmel SC, Levin RI, Martiniuk F, Weissmann G. A mechanism for the antiinflammatory effects of corticosteroids: the glucocorticoid receptor regulates leukocyte adhesion to endothelial cells and expression of endothelial-leukocyte adhesion molecule 1 and intercellular adhesion molecule 1. Proc Natl Acad Sci U S A 1992 Nov 1;89(21):9991-5.

22. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids--new mechanisms for old drugs. N Engl J Med 2005 Oct 20;353(16):1711-23.

23. Metselaar JM, Wauben MH, Wagenaar-Hilbers JP, Boerman OC, Storm G. Complete remission of experimental arthritis by joint targeting of glucocorticoids with long-circulating liposomes. Arthritis Rheum 2003 Jul;48(7):2059-66.

24. Wang M, Zhao D, Spinetti G, Zhang J, Jiang LQ, Pintus G, et al. Matrix metalloproteinase 2 activation of transforming growth factor-beta1 (TGF-beta1) and TGF-beta1-type II receptor signaling within the aged arterial wall. Arterioscler Thromb Vasc Biol 2006 Jul;26(7):1503-9.

25. Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. Circ Res 2002 Feb 22;90(3):251-62.

26. Sierevogel MJ, Pasterkamp G, Velema E, de Jaegere PP, de Smet BJ, Verheijen JH, et al. Oral matrix metalloproteinase inhibition and arterial remodeling after balloon dilation: an intravascular ultrasound study in the pig. Circulation 2001 Jan 16;103(2):302-7.

27. Meijer CA, Stijnen T, Wasser MN, Hamming JF, van Bockel JH, Lindeman JH. Doxycycline for stabilization of abdominal aortic aneurysms: a randomized trial. Ann Intern Med 2013 Dec 17;159(12):815-23.

28. Nieves Torres EC, Yang B, Roy B, Janardhanan R, Brahmbhatt A, Leof E, et al. Adventitial delivery of lentivirus-shRNA-ADAMTS-1 reduces venous stenosis formation in arteriovenous fistula. PLoS One 2014;9(4):e94510.

29. Pires NM, Schepers A, van der Hoeven BL, de Vries MR, Boesten LS, Jukema JW, et al. Histopathologic alterations following local delivery of dexamethasone to inhibit restenosis in murine arteries. Cardiovasc Res 2005 Dec 1;68(3):415-24.

30. Schepers A, Pires NM, Eefting D, de Vries MR, van Bockel JH, Quax PH. Short-term dexamethasone treatment inhibits vein graft thickening in hypercholesterolemic ApoE3Leiden transgenic mice. J Vasc Surg 2006 Apr;43(4):809-15.

31. Villa AE, Guzman LA, Chen W, Golomb G, Levy RJ, Topol EJ. Local delivery of dexamethasone for prevention of neointimal proliferation in a rat model of balloon angioplasty. J Clin Invest 1994 Mar;93(3):1243-9.

32. Reil TD, Sarkar R, Kashyap VS, Sarkar M, Gelabert HA. Dexamethasone suppresses vascular smooth muscle cell proliferation. J Surg Res 1999 Jul;85(1):109-14.

33. Roy-Chaudhury P, Spergel LM, Besarab A, Asif A, Ravani P. Biology of arteriovenous fistula failure. J Nephrol 2007 Mar;20(2):150-63.

34. Asif A, Roy-Chaudhury P, Beathard GA. Early arteriovenous fistula failure: a logical proposal for when and how to intervene. Clin J Am Soc Nephrol 2006 Mar;1(2):332-9.

35. Dember LM, Beck GJ, Allon M, Delmez JA, Dixon BS, Greenberg A, et al. Effect of clopidogrel on early failure of arteriovenous fistulas for hemodialysis: a randomized controlled trial. JAMA 2008 May 14;299(18):2164-71.

36. Zaragatski E, Grommes J, Schurgers LJ, Langer S, Kennes L, Tamm M, et al. Vitamin K antagonism aggravates chronic kidney disease-induced neointimal hyperplasia and calcification in arterialized veins: role of vitamin K treatment? Kidney Int 2015 Oct 14.

37. Kokubo T, Ishikawa N, Uchida H, Chasnoff SE, Xie X, Mathew S, et al. CKD accelerates development of neointimal hyperplasia in arteriovenous fistulas. J Am Soc Nephrol 2009 Jun;20(6):1236-45.

38. Kang L, Grande JP, Hillestad ML, Croatt AJ, Barry MA, Katusic ZS, et al. A new model of an arteriovenous fistula in chronic kidney disease in the mouse: beneficial effects of upregulated heme oxygenase-1. Am J Physiol Renal Physiol 2016 Mar 15;310(6):F466-F476.

39. Dwivedi AJ, Roy-Chaudhury P, Peden EK, Browne BJ, Ladenheim ED, Scavo VA, et al. Application of human type I pancreatic elastase (PRT-201) to the venous anastomosis of arteriovenous grafts in patients with chronic kidney disease. J Vasc Access 2014 Sep;15(5):376-84.