Experimental therapeutic strategies in restenosis and critical limb ischemia

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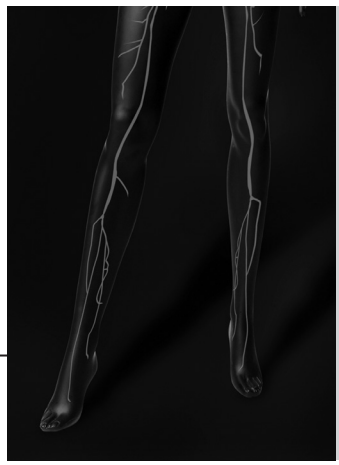

Chapter 09

Summary and Perspectives

Summary and perspectives

Several substantial clinical problems remain in vascular surgery despite of many technical advances. This thesis addresses two of those problems and is based on clinical research on new interventional approaches. The studies presented in the first part concentrate on the role of radiation therapy to reduce or to prevent (re)stenosis caused by intimal hyperplasia of the vessel wall after percutaneous transluminal angioplasty (PTA) of atherosclerotic disease and prosthetic arteriovenous (AV) fistulas for hemodialysis. The second part of this thesis focuses on the experimental therapeutic strategy of cell therapy in patients with severe limb ischemia who are without conventional surgical or endovascular treatment options.

The general introduction **chapter 1** presents the background of the clinical problems discussed in the thesis. The chapter provides an overview of the mechanisms of (re) stenosis after surgical or endovascular intervention, along with a review of the various strategies to control this process after balloon angioplasty and surgical reconstruction of AV fistulas.
Subsequently, the rationale of cell-based therapy in vascular surgery is discussed. It is part of an emerging field of vascular regenerative medicine whereby (new) blood vessel growth is induced in order to supply oxygen and nutrients to the ischemic limb.

Radiation for prevention of restenosis: The vanishing act of vascular radiotherapy
Chapter 2 reviews the concept of radiotherapy for inhibiting intimal hyperplasia and arterial remodeling in vascular surgery. The effect on proliferation of cells to ionising radiation has been extensively studied for the past four decades. When radiation is absorbed in biological tissue, it can directly ionise a critical site (direct effect) or interact with other molecules to produce reactive free radicals, which can subsequently damage critical biological molecules (indirect effect). DNA is considered the critical target damaged by ionising radiation by both direct and indirect processes. Since radiotherapy had proven to be effective in the treatment of non-malignant proliferative processes, e.g. keloid reduction, it was hypothesized that this adjunctive treatment would also inhibit vascular restenosis. Generally, radiation could be either applied externally or intravascularly. The major difference between external and intravascular radiation was dose distribution. Intravascular delivery resulted in extremely high doses to the vessel wall with a fall-off in dose as a function of distance from the source; whereas, external beam delivered a uniform dose over the entire volume of tissue treated. In case of intravascular delivery, both β and γ sources were used. Regarding the lower penetration properties and more heterogeneous distribution of β radiation, its use was restricted to smaller (coronary) vessels. Procedures with γ radiation (coronary and peripheral vessels) on the other hand required strict safety measures and were therefore more cumbersome. As radiation

has the potential to irreversibly injure blood vessels, the primary challenge in clinical vascular radiotherapy was to restrict its effect to inhibition of restenosis but also to avoid irreparable damage.

The results of our first clinical trial for the prevention of restenosis after femoropopliteal percutaneous transluminal angioplasty (VARA trial) are described in **chapter 3.** Sixty patients with symptomatic stenotic or totally occluding lesions in the femoropopliteal artery were randomised to be treated with PTA plus endovascular brachytherapy (EBT) or PTA alone. In case of EBT, 14 Gy was applied by an Ir^{192} source to the vessel wall. Eventually, 53 patients could be studied. After 12 months, restenosis rates of the treated segment by duplex ultrasound were 44% (12/27) in the PTA group versus 35% (8/23) in the PTA + EBT group (x^2 test, P=0.51). There was no difference in mandatory reintervention between the two groups. Overall, EBT resulted in an absolute risk reduction of significant restenosis of 9%, yet in patients with completely occlusive disease this reduction was 32%.

In **Chapter 4** we studied the effect of external beam irradiation (EBI) on the patency of prosthetic AV fistulas (AVF trial). Since the major cause for failure is thrombotic occlusion due to stenosis caused by intimal hyperplasia at the site the venous anastomosis, this site was experimentally treated. Fifty patients were randomly assigned to receive an 18 Gray postoperative dose in two fractions or to surgery only. Although no signs of any radiation related side effects were found after 12 months follow-up, radiation treatment did not result in less stenoses or reinterventions after radiation in polytetrafluoroethylene grafts for dialysis access but might even worsen patency rates. On an intention to treat base, the stenosis rate after 12 months by duplex ultrasound was 56% in the EBI group vs. 37% in the control group (log-rank test, p = 0.58). In the per protocol analysis, stenosis rates at the site of the venous anastomosis after 12 months were 66 and 37%, respectively (log-rank test, P=0.05). The primary patency rate, i.e., the fraction of functioning AV fistulas without intervention after 1 year of follow-up was 36% in the EBI group and 51% in the control group (log-rank test, P=0.29). Per protocol analysis showed a 1-year primary patency rate of 20% and 54%, respectively (log-rank test, P=0.04).

The outcome of the VARA trial related reasonably well with the results reported by others. Restenosis rates in the control group were relatively low, probably due to a substantial percentage of patients with low-complex stenoses in the study. In these cases the benefit of EBT might be less obvious. In AV-fistulas we could not demonstrate a beneficial effect of radiotherapy. Actually comparative data are scarce since work by others is mainly confined to animal studies and safety and feasibility reports.
These trials were published in 2003 and 2005 and since the field has made a significant turn in recent years it is appropriate to contemplate the fate of vascular irradiation. Its role should not only be considered within the scope of peripheral interventions

since by far the greater part of further development occurred in coronary arteries. Percutaneous transluminal coronary angioplasty has become the most frequently used method for myocardial revascularization. The use of uncoated coronary-artery stents during percutaneous intervention has decreased the incidence of acute complications and improved the outcome of patients, but in-stent restenosis (ISR) compromises the long-term results.[1] The efficacy of EBT in treating ISR was proven in the late 1990s in large randomised placebo-controlled trials for both intracoronary γ- and β-irradiation.[2-8] These studies demonstrated a sharp reduction in repeat revascularisation procedures with brachytherapy. Target vessel revascularisation (TVR) of ISR-lesions dropped from 30-60% to 15-30%. By the year 2000 brachytherapy had become an established technique for this indication.

At the same time the role of radiotherapy following balloon angioplasty in femoropopliteal arteries was established, to a great extent by the Vienna 1-5 trials.[9-13] After a pilot-study, the Vienna-2 trial showed a significant reduction of the restenosis rate with a 12 Gray dose after 6 months in 113 patients (table IV chapter 2).[10,14] Similar results were obtained after 12 months follow-up and a 18 Gray dose in the Vienna-3 trial.[11] Analysing both studies with stratification for de-novo or recurrent lesions, it became clear that EBT only significantly reduced restenosis after femoropopliteal angioplasty of recurrent but not de-novo lesions. At that moment, many questions were still unanswered. Which cells were the target for radiotherapy: injured cells in the intima, media or adventitia? Was β-radiation, which has clear advantage for radioprotection and hence for implementation in clinical practice, as effective as the γ-rays from the first trials? Which was the ideal dose to deliver? How to explain the "edge failures": was this a target volume or a dose distribution problem? Or could there be a stimulating effect of low dose irradiation on intimal hyperplasia?

Especially in the interventional cardiology, investigators took advantage of large patient numbers and industry investments. After a steady growth for a decade, around 2005 this rise was followed by a rather steep fall. This was caused by the apparent limitations mentioned above (only effective in recurrent lesions) and the very cumbersome nature of the procedure. The most consequential factor however, that probably formed the "death blow" for EBT was its limited long-term efficacy. Follow-up data from the first prospective randomised study (Vienna-2)[10] demonstrating significant short-term efficacy of EBT after PTA, showed a late catch-up phenomenon: after 5 years the recurrence rate was comparable in both groups.[15] The initial beneficial findings of EBT in other trials proved not to be sustainable over time as well.[16] (Studies are summarised in table IV, chapter 2).
Also in the coronary setting EBT did not seem favourable in the long run[17] and a new, more promising tool appeared on the restenosis horizon. In a short period of time, the coronary field was taken over by drug-eluting stents. Two important randomised multicenter trials demonstrated that treatment of in-stent restenotic lesions with drug-eluting stents rather than angioplasty followed by EBT, reduced clinical and angiographic restenosis after 1 year follow-up and improves event-free survival.[18,19]

While trials are ongoing, the use of drug-eluting stents in peripheral arteries should be considered experimental. The first reports indicate safety and feasibility; efficacy compared with bare-metal stents however is not yet convincing.[20]
In-stent restenosis after coronary angioplasty was the only FDA-approved indication for vascular radiotherapy at the time. For that reason it is not surprising that other possible indications like AV-fistulas were pulled along the downfall. However, since there is no lasting solution for the prevention of restenosis in AV-fistulas, proceeding the research as suggested in chapter 4, is not completely excluded.

Cell therapy for limb ischemia: experiment between hype and hope

The large unmet medical need of no-option patients – those with severely disabling claudication or critical limb ischemia who are not amenable to conventional endovascular or surgical treatment options – has propelled the development of "biological" revascularisation that focuses on the growing of new blood vessels. This field is emerging and a general overview of the current insights into mechanisms of vascular growth in the adult is given in **chapter 5**. In particular the role of angiogenic factors, the immune system, and bone marrow, are discussed, together with modes of its therapeutic stimulation and results from recent clinical trials.
Three concepts of vascular growth have been described to date, being angiogenesis, vasculogenesis and arteriogenesis, which represent different aspects of an integrated process. *Angiogenesis* involves the sprouting of new capillary-like structures from existing vasculature. The term *Vasculogenesis* is presently commonly used for the intussusception of bone marrow derived progenitors cells into the expanding vascular area whereas *arteriogenesis* is the development of adult collateral arteries from a pre-existing arteriole network. The three above described concepts of vascular formation probably all play a role in adult neovascularisation, and usually occur simultaneously at different levels. Stimulation of vascular growth seems clinically feasible. VEGF is the most extensively studied and crucial pro-angiogenic factor. In animal models of hind limb ischemia vascular growth is successfully promoted. Yet, results in placebo-controlled patient studies were less beneficial. Most recently transplantation of autologous bone marrow is attempted with some beneficial results, although the mechanism of action is far from complete understanding.
We performed a study after the safety, feasibility and potential efficacy of bone marrow cells in patients with advanced limb ischemia, described in **Chapter 6**. In 27 patients who were without technical options for revascularisation by percutaneous transluminal angioplasty (PTA) or reconstructive surgery, $1.23 \pm 0.49 \times 10^9$ autologous bone marrow-derived mononuclear cells (BMCs) were administered in a 40 mL concentrate. Delivery was either combined intramuscularly / intra-arterially (n=12) or exclusively intramuscularly (n=15). There were no adverse reactions related to injection of the cells. Three patients died within the first year of follow-up due to non-procedure related causes. Two patients

in the combined delivery group required limb amputation because of ongoing critical ischemia versus seven patients in the intramuscular group (P=0.17). BMC treatment in the remaining patients resulted in a significant and sustained (>12 months) improvement. Pain-free walking distance improved from 81 ± 56 meters at baseline to 257 ± 126 meters at t=6 months (P=0.0002). Mean ABI increased 23% after 6 months (P=0.01) and pain score reduced for up to 50% as shown by Brief Pain Inventory (P=0.001).
The angiographic results of this study and hypotheses on the clinical usefulness of digital subtraction angiography in the assessment of the effects on inducement of collateral arteries after cell-based therapy were separately considered in chapter 7. To date, in clinical studies, the emphasis has been on demonstrating recovery of clinical parameters, rather than the evaluation of blood flow improvement. The study comprised the angiographies of 16 patients since 9 of the earlier mentioned 27 patients underwent amputation and there were 2 deaths within the first 6 months. All patients were unilaterally treated with autologous bone marrow-derived mononuclear cells, pre- and six months post-treatment digital subtraction angiographies (DSA) were assessed and compared in a blinded fashion twice by a panel of seven vascular surgeons and interventional radiologists. Inter- and intraobserver variability on qualitative (poor/moderate/rich) and semi-quantitative (increase/no difference/decrease) assessment of collateral circulation were evaluated. Agreement was expressed as inter- and intraclass correlation coefficients (CC). Inter- and intraobserver agreement was moderate for the qualitative grading of collateral extent (CC=0.46 and 0.60 respectively). Agreement was moderate (inter-CC=0.60) to good (intra-CC=0.73) for comparing pre- and post-treatment DSA. No difference was found in the extent of collateral formation comparing both DSA after separate analysis of clinical responding and non-responding patients (P=0.92).
The way that the results of both studies described in chapter 6 and 7 relate to the results of others is discussed in the respective chapters.

DSA is regarded as the gold standard for anatomical assessment of vascularization in PAOD. Measuring the pressure in the ankle arteries however has become the standard in the initial evaluation of patients with suspected peripheral arterial occlusive disease (PAOD). Ankle-brachial pressure index measurement (ABI) provides functional information about the presence and severity of PAOD. Patients with diabetes however, may have stiffer or even densely calcified arteries that are less or even not compressible resulting in falsely elevated and meaningless ankle pressures. Measurements therefore may underappreciate disease severity and renders ABI of limited use in a large group of patients with diabetes. Therefore we evaluated a new functional test for the assessment of PAOD in patients with diabetes mellitus in **chapter 8**.
The diagnostic accuracy of the Doppler derived maximal systolic acceleration (ACC_{max}) was evaluated in a retrospective analysis. ACC_{max} was measured at ankle level in 163 consecutive patients referred to the vascular laboratory for initial assessment of PAOD. ACC_{max} values

were compared with the ABI reference standard. Patients were classified according to the presence or absence of diabetes. In the non-diabetic patients PAOD was defined as ABI ≤0.90. This group was used to establish the association between ACC_{max} and ABI in a linear regression model. The result was then used to predict PAOD in the diabetic patients. A total of 301 lower limbs were examined. The study group consisted of 166 limbs of patients without diabetes and 135 limbs of patients with diabetes. PAOD was present in 52% of limbs in the nondiabetic group versus 59% of limbs in the diabetic group (ABI ≤0.90, or in case of non-compliant vessels toe-brachial index (TBI) ≤0.70). An ACC_{max} cut-off value of >10 m/s^2 was found to be highly predictive for the exclusion of PAOD (negative predictive value 95%). In addition, the ACC_{max} cut-off value of <6.5 m/s^2 was highly predictive for the detection of PAOD (positive predictive value 99%). A strong quadratic association was found between ACC_{max} and ABI in the non-diabetic group (r^2=0.85). In the diabetic patients r^2 values were 0.81 and 0.79 after ABI and TBI measurement respectively.

Final remarks

The concept of cell therapy originated from the hypothesis that supply of a cocktail of cytokines, growth factors and cells to ischemic tissue could be a more physiological and effective approach of achieving vascular growth. Most trials made use of the mononuclear cell fraction from the bone marrow. Although the feasibility of this approach has been shown in several animal models and preliminary clinical studies, we still don't know very much known about the working mechanism and clinical effectiveness of cell therapy. Actually, much is based on hypotheses. Future basic research should focus on elucidating the mechanism and clinical research should focus on efficacy, durability, safety and optimal delivery mode. Current topics in the context of the above mentioned issues are the following:

Ideal cell type

Stem cells are defined as undifferentiated cells with the capacity for proliferation and self renewal, as well as an ability to regenerate multiple cell types and tissues. Embryonic stem cells are derived from mammalian embryos and have the ability to generate any differentiated cell in the body. Adult stem cells are part of the tissue-specific cells of the organism into which they are committed to differentiate.[21] They have been isolated from various tissues as the bone marrow, peripheral blood, umbilical cord, central nervous system and liver.

In summery, the ideal cell population for stimulating vascular growth would:

- be able to differentiate into mature stages of different tissue types and regenerate themselves without losing their differentiation potential.
- generate new vasculature, regardless of the mechanism. Preferably large-caliber conducting vessels, since capillaries only are insufficient to increase flow sufficient to improve tissue perfusion.
- be highly resistant to ischemia and apoptosis

- be of autologous origin
- be easily available in large quantities

Ideal cell delivery

Until now, no delivery strategy has come forward as the most optimal route in limb ischemia: direct intramuscular injection, intra-arterial application or a combination of both? We discussed the rationale of combined delivery in chapter 6. Our study showed no difference between intramuscular and combined intramuscular and intra-arterial cell delivery, but was not primarily designed for this purpose.[22]

An important aspect is the retainment in the target-tissue of transplanted cells. This so-called homing process is dependent on the local milieu and homing signal may vary in different clinical circumstances. In chronic limb ischemia, the presence of homing signals e.g. chemoattractants and cell adhesion molecules, seems less outspoken.[23] Assessment of tissue distribution demonstrated that, depending on the delivery method, only a few percent of the injected bone marrow cells (BMCs) could be traced in the ischemic tissue. The vast majority of the cells was localised in the liver and spleen of the animal models.[24] Possible strategies to improve homing of the cells will be another subject of research.

Ideal cell function

Patient factors, such as age, diabetes and hypercholesterolemia are associated with an impaired endogenous response to ischemia and are also associated with reduced numbers and function of circulating stem cells.[25] In general, patients qualifying for stem cell therapy have end-stage disease and have failed multiple prior conventional revascularisation attempts. These particular individuals may thus be poorly suited to respond to pro-angiogenic therapies. Besides, a large proportion of the transplanted cells will undergo apoptosis after a few days.[26] Future approaches to therapeutic angiogenesis may combine experimental strategies. Genetic modification of stem cells could overexpress angiogenic growth factors to enhance the signaling activity and rejuvenate the bioactivity and/or extend the lifespan of the cells.

Possible adverse effects

Discussing the potential for cell-based therapy in patients with peripheral arterial disease, one must also consider the potential drawbacks of angiogenesis, that is, the identification of factors augmenting tumor neovascularisation such as VEGF.[27] Likewise, progenitor cells can incorporate in tumor vasculature and may contribute to tumor vascularisation.[28] For the same reason, stimulation of vascular growth may also theoretically result in retinopathy and the progression of atherosclerosis.[29] Accordingly, attempts to induce neovascularisation have raised some concerns regarding the possibility of inducing unwanted or pathological vessel growth. On the other hand, a growing body of evidence suggests that circulating stem cells play an important role in endothelial cell regeneration

plus reducing atherosclerotic plaque size and might therefore rather prevent than provoke atherosclerosis.[30]
Thus far, with numerous patients in various trials of peripheral and cardiac cell-based therapy, there has not been an indication of increased incidence of malignancies, retinopathy or acceleration of atherosclerosis.

Mechanism
Given the plasticity of various bone marrow-derived cell populations (Chapter 5, figure 2), it is tempting to suggest that cell-based therapy enhances neovascularisation by direct incorporating into the vessel wall.[31,32] However, data on this transdifferentiation of these cells into new endothelial cells are also conflicting. Others challenged this theory with convincing evidence that BMCs do hardly, or not at all, incorporate and that vascular growth is promoted by a paracrine effect of these cells. Bone marrow cell populations contain very small number of stem cells, <0.01% of total cells. Since many bone marrow subpopulations are a source of growth factors, cytokines and chemokines, a complementary hypothesis is that the cells act in a more supportive role.[33,34]
Currently, the main believe is that the potentially beneficial action of BMCs is related to paracrine release of growth factors but not to transdifferentiation.

The ultimate success of cell therapy however will rest on its ability to demonstrate clinical efficacy rather than on an attributed complex mechanism. In fact, the understanding of many other mechanisms of many other therapeutics in medicine has been provisional. Identifying which of the cellular components is necessary for beneficial effects, and whether these effects are mediated directly by the transplanted cells or indirectly through involvement of other cells, would enable targeted delivery of essential components. This is likely to be a critical step in the full realisation of the potential of this therapeutic approach. Therefore, one should not expect too much too soon and maybe cell-based therapy seems even more remote than it did 5 years ago.

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