PREDISPOSANT FACTORS FOR INTIMAL HYPERPLASIA AND THEIR MECHANISMS OF ACTION IN OPEN VASCULAR SURGERY. A REVIEW

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PREDISPOSANT FACTORS FOR INTIMAL HYPERPLASIA AND THEIR MECHANISMS OF ACTION IN OPEN VASCULAR SURGERY. A REVIEW (Abstract): Background: Intimal hyperplasia is a multifactorial process that determines local stenosis and subsequent ischemia after revascularisation procedures. It is controlled by a plethora of cytokines, growth factors and proteases that interact through multiple mechanisms, empowering or inhibiting their hyperplasic action. Although the elements involved are the same, the resulted degree of vascular stenosis varies. Methods: To highlight the predisposing conditions which enhance the vascular response to injury we consulted articles from Pubmed database, searching for the keywords "intimal hyperplasia", "neointimal hyperplasia" and "risk factor". Results: 101 articles were included in the study. Risk factors analyzed are classified into local and systemic and the mechanisms by which they augment the process are explained. Conclusion: Knowledge of risk factors and their control when possible is a key element in increasing revascularization patency. Keywords: VASCULAR INJURY; INTIMAL HYPERPLASIA; RISK FACTORS.

Intimal hyperplasia, a process triggered by vascular injury, is the proliferation of vascular smooth muscle cells in the media, followed by their migration to the intima, the transformation from contractile in secretory phenotype and secretion of extracellular matrix constituents, end result being a narrowing of the vascular lumen and subsequent ischemia in the irrigated territory (1). The process is controlled by a plethora of cytokines, growth factors and proteases that interact through multiple mechanisms, empowering or inhibiting their hyperplasic action (1). Although the elements involved are the same, the resulted degree of vascular stenosis varies. This raises the question whether there are predisposing conditions which is thought to enhance the vascular response to injury and can be controlled in order to increase revascularization patency.

METHOD
Consulting articles from the Pubmed database, we conducted a search for the combination of keywords "intimal hyperplasia" AND "risk factor", “neointimal hyperplasia” AND "risk factor", until september 2014. The search yielded 430 articles. The two authors individually screened the abstracts and 101 were found relevant for this study. From this articles, the fol-
Predisposant factors for intimal hyperplasia and their mechanisms of action in open vascular surgery. A review

Following variables were collected: paper first author, year of publication, risk factor described, and method of determination (animal experiments, clinical trials).

RESULTS

Although there is a great heterogeneity of the determined risk factors, for a better understanding we decided to classify into local and systemic factors. For every risk factor both experimental and clinical trials results are reviewed and a short description of the mecanism involved is provided.

LOCAL FACTORS

Local hemodynamic factors have a major influence on vascular remodeling process. A basic conditions for an increased patency in arterial revascularization is the existence of a quality runoff. Christoph Hehrlein conducted an experiment on dogs, ligating the femoral artery proximal to its trifurcation and thus altering runoff and then producing laser thermal injury and balloon endothelial denudation. He found an average increase of 32% of the area of intimal hyperplasia in animals with altered runoff compared to control, p <0.01 (2), independently of the severity of the lesion. The trend of development of neointimal tissue in conditions of poor runoff was explained by Dobrin. The author has quantified the effect of each force acting on the vascular wall in subsequent histological changes and found that intimal hyperplasia occurs under low shear stress conditions (3). Shear stress is directly proportional to the speed of blood flow, which in turn is directly influenced by the runoff. Bassiouny showed that at the level of an end-to-side anastomosis there are two areas of intimal hyperplasia: at the suture line and at the receiving vessel floor. Realizing an artificial model which simulated the conditions of geometry and pulsatility of an end-to-side anastomosis, he showed that at the floor level there is an area of stagnant flow compared to the cuff, where the flow rate is high and it correlates with the lack of cuff hyperplasia experimentally demonstrated (4). Another way in which an altered runoff promote intimal hyperplasia is due to a low local concentration of vasodilator endothelium released factors (NO, prostacyclin). As Rubanyi proved, the release of this factors is directly proportional to the flow rate (5). These substances have a powerful antimitogenic effect. Under conditions of poor runoff, their low concentration allows the unduly development of neointimal tissue. In this regard, Morinaga showed that intimal hyperplasia areas shrinks if the vessel is reimplanted into a normal flow parameters system (6). All of this experimental studies were confirmed by clinical trials. In a series of 191 femoropopliteal and femorodistal reconstruction, it was determined that the rate of patency at 6 months dropped from 88.2% in patients with good runoff to 21.8% in those with altered runoff (p<0.01) (7). Scali shown that a low distal graft end-diastolic velocity determined by duplex ultrasound at the end of the procedure predicted poor outcome in terms of patency (8).

Characteristics of autologous grafts.

Vein grafts used for myocardial and peripheral revascularization often show areas with altered histological structure, either fibrous or varicose. Histological studies showed in these areas a severe medial disruption, with smooth muscle cells transformation from contractile to secretory phenotype, an increase in the number of vasa vasorum and altered extracellular matrix metabolism (9). Transplantation of
venous grafts in the arterial circulation submit them to a tensile and shear stress much higher than those normally acting on the vein wall. Normal physiological response to the augmentation of these forces is the process of "arterialisation", characterized by pannmural hypertrophy, both cellular and matrix (9). This process is triggered by endothelial denudation caused by shear stress and muscle cell proliferation and subsequent apoptosis caused by tensile stress (10). Both in stenotic and varicose dilated areas laminar flow is affected, there are major turbulence and tangential deformation which causes major endothelial and medial damage (11). These lesions, appeared on a vascular wall with altered histological structure, have the effect of an exacerbated intimal and medial hyperplasia. It was shown experimentally that external stenting of venous grafts resulted in uniform flow, attenuation of tangential distension and limited intimal proliferative response (12). This procedure was adopted in clinical practice. A randomized trial of external stenting for saphenous vein grafts in coronary artery bypass grafting found that mean intimal hyperplasia area was significantly reduced in the stented group versus nonstented group and determined marginally significant improvement in lumen uniformity and less ectasia in the stented group (13).

Vascular trauma occurred during the process of harvesting the graft is another factor contributing to the development of neointima. It has two components. First, the dilatation has the effect of physical trauma - endothelial denudation. In these areas there is adherence of platelets and leukocytes that release cytokines and growth factors (14). Bare areas are endotelized in the first 14 days by prolifera-
who have insulin resistance as well in patients receiving intermittent subcutaneous insulin (22). Insulin stimulates intimal hyperplasia by accelerating the production of growth factors and by direct mitogenic effect (23). The second cause is represented by the advanced glycation end products (AGEs), which accumulate in vascular tissue with age (24). The vascular smooth muscle cells have specific receptors (RAGE) for them and the AGE - RAGE interaction results in stimulation of inflammation, smooth muscle cell proliferation and increased secretion of extracellular matrix components (25). In a murine model, insulin-resistant Zucker fatty rats who received long acting insulin therapy for 4 weeks, carotid intima/media ratio was significantly reduced after balloon catheter injury compared to control (26). A study performed on 14,788 patients who underwent infrainguinal lower extremity arterial bypasses found that diabetes mellitus had a negative association with early graft failure (95% confidence interval, 0.58-0.89, p=0.002), determined by intimal hyperplasia and not by progression of atherosclerosis (27).

Additive effect of hypercholesterolemia in the development of postlesional intimal hyperplasia is known since 20 years ago and is based on exacerbating local inflammatory process (28). Under conditions of hypercholesterolemia, monocyte chemoattractant protein (MCP-1), also known as chemokine ligand 2 (CCL2) is expressed on vascular smooth muscle cells in large numbers (29) and its receptor (CCR2) on the surface of monocytes. CCL2-CCR2 interaction is the main way by which monocytes are recruited in increased number at the site of an endothelial injury (30), maintaining the inflammatory process and stimulating the development of neointimal tissue. The inhibition of this interaction by administration of CCL2 monoclonal antibodies has resulted in a marked reduction in neointimal area (31). In animal experiments it was found that under hypercholesterolemia, there is a growing mRNA expression of type I collagen, increased secretion of this type of collagen in the extracellular matrix, together with a significant reduction of matrix metalloproteinase activity (32). A third negative effect induced by hypercholesterolemia, closely linked to oxidative stress, is the slowdown in postlesional reendothelisation process (33). In a study on patients with carotid restenosis after endarterectomy, it was found that levels of plasma cholesterol, total triglycerides and low density lipoprotein apoprotein B were higher than control (34).

Homocysteine. One of the first studies that have shown a link between increased blood levels of homocysteine and postendarterectomy progression of carotid intimal hyperplasia was carried out on a animal model by Frederick Southern in 1998 (35). Since then, many of the mechanisms by which hyperhomocysteinemia causes vascular injury and promotes neointimal formation were elucidated and are represented by chronic inflammation and subsequent endothelial damage, modulation of gene expression by altering methylation, inducing a procoagulant status, induction of apoptosis and extracellular matrix remodeling (36). The possibility of hyperhomocysteinaemia being a risk factor for vein grafts stenosis was examined in a case-control study, that revealed a direct connection between elevated plasma levels of homocysteine and low patency rates (37).

Coagulation disorders. Malfunctio
mented vascular hyperplasia. Platelet adhesion to vascular lesion and parietal thrombus formation are the triggers for the vascular healing process. It is known the role of platelets in the process, mainly through PDGF release, the most powerful mitogen for cells of mesenchymal origin and a potent chemotactant for smooth muscle cells, macrophages and neutrophils (38). An exacerbated response to injury when the platelet count is pathological increase was documented in chronic myeloproliferative disorders, especially polycythemia vera and essential thrombocythemia and is manifested both in the medium vessels - the coronary and pedal arteries (39) and the smaller vessels - arteries dermis, causing generalized racemose livedo (40).

Factor Xa forms a complex with factor Va and cleaves prothrombin to active thrombin, which converts fibrinogen to fibrin (41). Thrombin also stimulates platelet aggregation and degranulation by cleaving protease activated receptors on the platelet membrane. It is a powerful smooth muscle cells mitogen and promotes their migration in the intima (42). Increased thrombin generation conditions, like neoplastic diseases, prothrombin thrombophelia or postmenopausal hormone replacement therapy were associated with an accelerated intimal hyperplastic response (43,44). Procoagulant activity of thrombin is specifically inhibited by antithrombin III in plasma and heparin cofactor II (HCII) in the subendothelial layer of the vascular wall, in the presence of dermatan sulfate. Because heparin cofactor II / dermatan sulfate complex effectively inactivates surface-bound thrombin, it inhibit proliferation of vascular smooth muscle cells and fibroblasts. A genetic deficiency of this factor is highly associated with an exacerbated response to injury (45). Takamori demonstrated on a group of 134 patients that a high HCII plasma level is a protective factor against in-stent restenosis (46).

Hyperplasia is also promoted when there is an increase in fibrinolysis. Plasmin is formed from plasminogen under the action of a series of activators (PA), most important being urokinase type – PA (u-PA) in the serum and tissue type – PA (t-PA) bound to fibrin (47). The role of plasmin is to remove the clot by degrading fibrin, but it promotes hyperplasia by mediating the migration of smooth muscle and inflammatory cells within the blood vessel wall, increasing collagen deposition and cholesterol accumulation (48). Plasminogen activator inhibitor type 1 (PAI-1), a protein synthesized by vascular endothelial and smooth muscle cells, is the primary inhibitor of t-PA and urokinase (49). Although increased levels of this inhibitor correlates with myocardial infarction (50), decreased plasma levels of PAI-1 is documented to be a risk factor for stent restenosis (51) and experimental animal models have shown to reduce the area of hyperplasia when the production of this protein is stimulated (52). These apparently paradoxical results have a common denominator, namely reduced production of plasmin. A low level of plasmin prevent fibrinolysis, thus promoting clot progression, but also this low level involves a decreased prohyperplastic effect.

**Cigarette smoking**

Known as the main risk factor in the progression of atherosclerosis, smoking plays an important role in initiating and augmenting intimal hyperplasia. Exposure to cigarette smoke increases the number of platelets and their ability to adhere to damaged vessel wall and inhibit their mito-
Predisposant factors for intimal hyperplasia and their mechanisms of action in open vascular surgery. A review

Mitochondrial activity (53). Smoking also cause neutrophilia and the activation of neutrophils by the complement system. Although neutrophils do not secrete growth factors, they contribute to tissue injury through the release of oxygen radicals and proteases (54). Chronic cigarette smoking increases monocyte adherence to cultured human umbilical vein endothelial cells in vitro, promoting inflammation (55). Endothelial cells are also affected, smoking disrupting normal regulatory properties, increasing cell wall permeability and the likelihood of detachment with injury and decreasing endothelial cell DNA synthesis. The smooth muscle cells are direct affected by cigarettes via polycyclic aromatic hydrocarbons found in the smoke, which are increasing their proliferation rate and viability (56). Experiments performed on murine models had shown that smoking accelerates intimal hyperplasia in a dose-dependent manner (57).

**Endothelial progenitor cells**

The vascular endothelium is a dynamic organ, formed by a continuous layer of cells that cover the luminal surface of blood vessels and react to physical forces and chemical signals, affecting vasomotion, thrombosis, platelet aggregation and inflammation. Endothelial progenitor cells (EPCs) isolated from peripheral blood are originating from the bone marrow. Their role is to migrate, differentiate into endothelial cells and cover intimal defects (58). It has been demonstrated a direct correlation between the decreased number of EPCs in peripheral blood and progression of coronary artery disease (59). Likewise, in laboratory experiments, autologous EPCs transplantation enhanced the vasculoprotective properties of the reconstituted endothelium resulting in inhibition of neo-intimal formation (60). Another study showed that anti-vascular endothelial cadherin antibody-coated stents captured circulating EPCs, thus resulting an accelerated endothelial recovery on stent and reduced intimal hyperplasia (61). The number of circulating EPCs falls in patients with hypercholesterolemia, vitamin D deficit, low plasma estrogen level and is stimulated by exercise training, administration of erythropoietin and statins.

**Discussion**

Although the progression of atherosclerosis in some cases is causing stenosis after open surgical revascularization, most frequently the low patency of these procedures is due to a compounded vascular healing process after injury. Therefore the vascular surgeon must know the intimate mechanisms by which the process unfolds and especially the causes that exacerbate it. The most important contribution that the doctor can have in limiting the negative effects caused by hyperplasia is represented by run-off selection, when this option exists. Whether it is coronary or peripheral revascularization, a poor run-off have catastrophic effects on intervention patency. For infragenicular bypasses, American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines recommend that the tibial or pedal artery that is capable of providing continuous and uncompromised outflow to the foot should be used as the site of distal anastomosis (62). This recommendation points out the importance of the outflow artery status throughout its length on limb salvage, patency and patient survival. There are also numerous angiographic scores that quantify the quality of runoff, the most used being Rutherford revised score, a method validated by clinical studi-
ies to predict the evolution of revascularization in patients with critical limb ischemia (63).

Vein graft harvesting, preparation and ischemia time also influence the degree of progression of intimal hyperplasia. Increasingly more surgeons are using the „no touch” technique in venous graft preparation. This procedure involves that the vein is neither stripped nor distended, but harvested with a fat pedicle. The PATENT study compared markers of vascular injury and smooth muscle cells activation in veins harvested using this technique vs the conventional one and found that „no touch” preserved intimal, medial and adventitial architecture, with similar leg recovery in both groups. (64). Distention during preparation is an important element in future graft evolution. A distention pressure lower than 140 mmHg prevents endothelial denudation, reduction of endothelial-dependent relaxation and intimal thickening and limiting intraluminal pressure can be obtained by using a pressure release valve (65).

Preparing the arteries before vein harvesting and making the anastomoses simultaneously by two teams of surgeons decreases graft hipoxia, simple gestures with a huge impact on long term patency. Many vascular surgeons refuse to use varicose veins in arterial reconstructions, preferring synthetic materials, but new external scaffolding technologies are emerging with encouraging results, especially in coronary surgery. External stenting stabilizes flow parameters, particularly shear stress, down-regulating the molecular processes involved in neointimal formation (66).

Although local factors outweigh the process, should not be forgotten the importance of systemic factors, which are most often controllable. The antidiabetic and lipid-lowering medication should be carefully determined and adjusted by the diabetologist prior to surgery and a close collaboration between the vascular surgeon and the haematologist is necessary in patients with myeloproliferative disease requiring revascularization procedures. New evidences emerge in favor of short-term dietary restriction immediately before surgery to reduce intimal hyperplasia and to protect from ischemia-reperfusion injury (67). Also, animal studies and in vitro experiments suggests that a short anti-inflammatory treatment with dexamethasone decreases vein graft thickening and levels of proinflammatory cytokines associated with hypercholesterolemia, but there is a lack of bed-side evidence (68). The beneficial effect of smoking cessation on reducing neointimal formation has been proven by numerous studies, so the patient should be directed to specialized antismoking centers prior to surgery. One study showed that stopping smoking rapidly increases circulating levels of endothelial progenitor cells and the magnitude of increase was greater in light smokers than in heavy smokers (69). Another study determined that heavy smoking before surgery significantly increase matrix metalloproteinase subtypes MMP-2 and MMP-9 gene expression, in the same time decreasing the gene expression of tissue inhibitors of metalloproteinases TIMP-1 and TIMP-2 (70).

All these therapeutic measures conducted at the right time can significantly increase the patency of revascularization procedures.

CONCLUSIONS

Intimal hyperplasia is a multifactorial process that determines local stenosis and subsequent ischemia. In addition to drugs
Predisposant factors for intimal hyperplasia and their mechanisms of action in open vascular surgery. A review

that have proven useful in inhibiting the process, knowledge of risk factors and their control when possible is a key element in increasing revascularization patency.

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1073


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