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Chapter 2

Future potential biomarkers for post-interventional restenosis and accelerated atherosclerosis

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Abstract

New circulating and local arterial biomarkers may help the clinician with risk stratification or diagnostic assessment of patients and selecting the proper therapy for a patient. Additionally they may be used for follow-up and testing efficacy of therapy, which is not provided by current biomarkers. Processes leading to post-interventional restenosis and accelerated atherosclerosis are complex due to many biological variables mediating the specific inflammatory and immunogenic responses involved. Adequate assessment of these processes requires different and more specific biomarkers. Post-interventional remodeling is associated with cell stress and tissue damage causing apoptosis, release of damage-associated molecular patterns (DAMPs) and upregulation of specific cyto/chemokines that could serve as suitable clinical biomarkers. Furthermore, plasma titers of pathophysiological process-related (auto)antibodies could aid in the identification of restenosis risk or lesion severity. This review provides an overview of a number of potential biomarkers selected on the basis of their role in the remodeling process.

Introduction

The current concept that inflammation plays a key role in the development of (post-interventional) atherosclerotic vascular remodeling has led to the investigation of inflammatory factors to serve as biomarkers for cardiovascular risk prediction. Multiple local arterial and blood-based biomarkers have been identified and selected for their association with a more adverse cardiovascular risk profile independently of known traditional risk factors such as dyslipidemia, hypertension, diabetes and smoking. Many of these markers have been incorporated into risk prediction models to improve risk assessment accuracy in addition to current diagnostic strategies[1]. Of these, C-reactive protein (CRP) is currently the best-validated inflammatory biomarker. Despite the value of plasma lipoprotein profiling and CRP measurements the picture is not yet clear. Many patients but not all continue to develop vascular remodeling following revascularization procedures and ongoing investigations into newer and more accurate or combined biomarker risk profiles remain necessary[2]. This review retracts the underlying pathophysiology of atherosclerosis and post-interventional vascular remodeling and the value of recently discovered inflammatory biomarkers[3] in the prediction of cardiovascular events in the biological context that require target lesion revascularization, highlighting their potential clinical value. Other biomarkers including genetic differences such as polymorphisms are not taken into account in this review.

Background of atherosclerosis and restenosis

Native atherosclerosis

Atherosclerosis is a chronic inflammatory disease of the large and medium-sized arteries and is initiated by a qualitative change in the endothelial monolayer by irritative stimuli such as dyslipidemia, hypertension, and pro-inflammatory mediators that lead to the exposure of adhesion molecules and infiltration of circulating leukocytes into the arterial wall[4]. Such mediators could be of high value when measured as biomarkers of lesion progression and stage of severity. Changes in endothelial permeability provoke the retention of cholesterol-containing low-density lipoprotein (LDL) particles that are endocytosed by monocytes-derived macrophages leading to foam cell and atheromatous lesion formation in the arterial tree[5, 6]. Tunica media-derived smooth muscle cell (SMC) migration and proliferation and extracellular matrix deposition lead to the formation of a fibrous cap overlying a necrotic core due to inefficient efferocytosis[5, 7]. Physical disruption of the plaque exposes the underlying thrombogenic material to the circulation triggering thrombosis formation, that may be monitored as biomarkers, and luminal occlusion with progressing ischemia in distal tissues[6], eventually leading to infarction requiring angioplasty or bypass-grafting[6, 8].

Post-interventional restenosis

Restenosis following angioplasty and stent implantation has been the major problem limiting the success rate of coronary interventions and tremendous efforts have been made to target this problem[9]. Acute and long-term vessel occlusion requiring target lesion revascularization following balloon angioplasty occurred in 30-60% of all pa-

tients due to elastic recoil and negative remodeling. The introduction of bare-metal stents (BMS) prevented elastic recoil and has reduced this incidence of restenosis to 16-44%, but also led to the development of neointimal hyperplasia[10]. Drug-eluting stents (DES) have been developed to counter this phenomenon, although incidence rates of 5-10% of in-stent restenosis (ISR) are still reported, encompassing over 200.000 revascularizations annually in the United States alone[11].Inflammation has been shown to be the driving factor behind these remodeling processes, pointing to a role as biomarker for this factor in the analysis of disease progression. DES have been successful in the prevention of neointimal hyperplasia, but have not been able to completely prevent the process of ISR. These figures support the need for development of new biomarker assays that allow careful screening of patients at risk for restenosis.

Restenosis is defined as more than 50% luminal loss at follow-up angiography with clinical restenosis defined as recurrence of symptoms such as angina pectoris or ischemia at rest, requiring repeat revascularization[12.] It has been proposed to be the result of an overshooting healing response that originally occurred in three distinctive phases: early loss due to elastic recoil, which occurs within minutes and has been successfully countered by the application of intracoronary stenting, followed by neointimal hyperplasia and eventually accelerated atherosclerosis development[13].
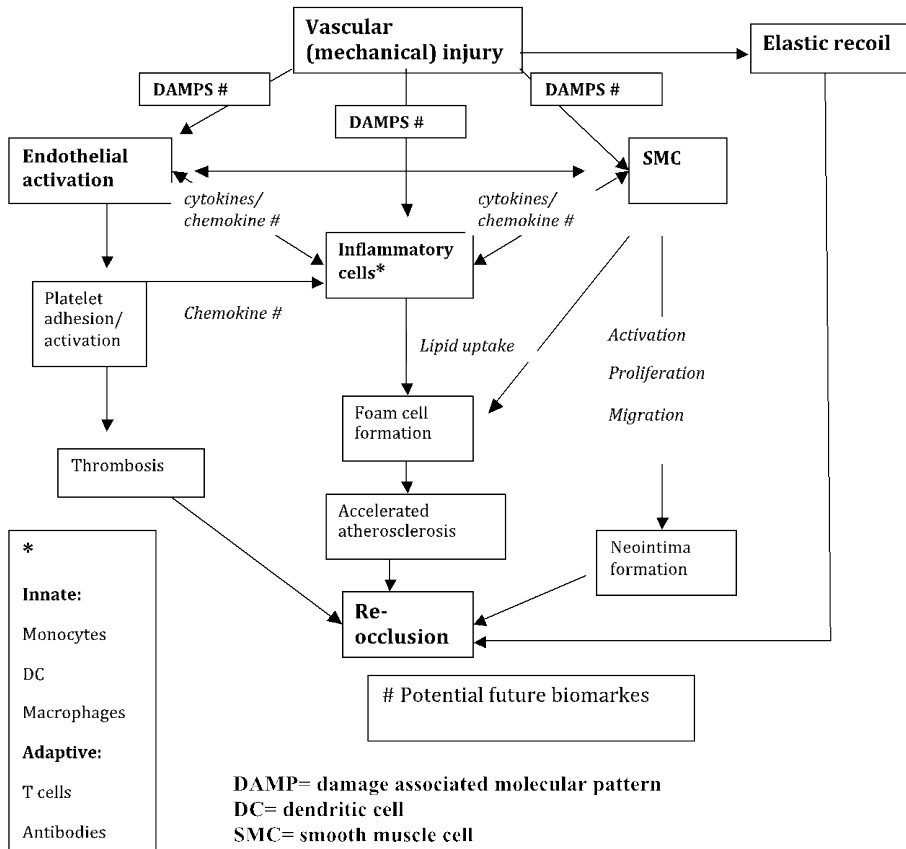

Figure 2.1 Post-interventional restenosis development

Neointimal hyperplasia is evoked by injury to the endothelium and underlying athero-sclerotic plaque, with exposure of thrombogenic content to flowing blood, supporting the adhesion and activation of thrombocytes and thrombosis. Platelets release mitogens, which can be traced throughout the plasma to serve as biological markers of thrombosis extent, and promote SMC migration and proliferation to the tunica intima with local extracellular matrix deposition[14]. This process is prevented or delayed (e.g. by many years) by DES compared to BMS implantation through the release of drugs that affect SMC migration and proliferation. This process is ultimately followed by a phase of vascular remodeling, in which accelerated atherosclerosis and concentric adventitial compression together further comprise lumen patency[13].

Underlying causes of restenosis

The underlying causes of restenosis can be divided into four general causes, namely biological, arterial, stent and implantation factors[10, 12]. Biological factors encompass the natural (genetic) vascular wall resistance to anti-proliferative drugs and the development of a sustained hypersensitivity reaction directed towards the polymer or metallic stent platform. Additionally, the initial levels of proteinases that determine SMC proliferation and migration are of great importance to treatment success[10]. These effects of proteinase of SMC proliferation and migration may be direct or indirect effects. In vascular remodeling matrix metalloproteinases may regulate migration, proliferation, and death of vascular smooth muscle cells by degrading matrix and non-matrix substrates[15], but also may play a role in activating other factors such as growth factor or other pericellular proteases[16]. Arterial factors that influence the vascular response are comprised of factor regulated by local wall shear stress levels, the progression of original atherosclerosis lesions growth within a stented segment, but also previous positive vascular remodeling. Stent factors that contribute to the development of ISR are the specific type of coating used, drug concentration and sustained period of release and to a lesser extent the drug of choice[17, 18]. Differences between effectiveness rates of specific drugs are determined by their ability to meet the biological threshold that exists and determines the initiation of an inflammatory response and the eventual occurrence of neointimal hyperplasia, which could be tracked with biomarker levels in plasma[10]. The stent gap, strut thickness and possible polymer disruption or cracking and eventual fractures are all important for proper stent effectiveness in the prevention of ISR[10]. Finally, technical implantation factors can limit therapeutic effectiveness that stenting could offer, such as an incomplete stent expansion and geographical misses, where the stent is deployed short of or beyond the complete lesion area[12]. During every interventional procedure the eventually of barotrauma to unstented segments and the deployment of a DES in a clot-laden arterial segment remains, that raise the chance of ISR after discontinuation of anti-thrombotic drugs. These factors support the search for biomarkers that could offer diagnostic insight at the time of procedure. These markers should be an adequate reflection of acute vessel injury.

Inflammation status as biomarker

The overall requirement of a cardiovascular disease biomarker is to enhance the ability to optimally manage the patient, identification of patients, to differentiate

patients, assess the likelihood of a therapeutic response, the risk of future recurrences and progression of disease[19]. The use of BMS or DES may have different effects on the pathophysiological process initiated and thereby on the inflammatory response and eventual potential biomarkers related to restenosis or in-stent thrombosis. For potential novel biomarkers it would be of major importance to be easily detectable and that levels correlate with disease progression. Since inflammation is importantly involved in vascular disease many studies focused on CRP as a biomarker. CRP is a strong marker of inflammation and upregulated in response to pro-inflammatory cytokines. No association was found for major cardiovascular events and high sensitivity-CRP, IL6 or TNFα by Sukhija and co-workers[20]. However, CRP seemed to be an excellent marker for post-stenting inflammation since it was produced mostly in response to pro-inflammatory cytokines released as a response upon the vascular damage initiated by the procedure[1]. In addition pre-procedural serum CRP-level proved to be an independent predictor of adverse outcome after coronary stent implantation, suggesting that a systemically detectable inflammatory activity is associated with proliferative responses within successfully implanted stents[21]. Higher baseline CRP levels of patients undergoing BMS implantation are a predictor of restenosis[22]. Interactions were also found between CRP levels, statin treatment and restenosis-incidence[23]. No clear association of CRP and restenosis was found during application of drug-eluting stents[1]. A study on first cardiovascular events and death based on the Framingham Offspring Study by Wang and co-workers showed that the most informative circulating biomarkers for predicting death proved to be B-type natriuretic peptide, C-reactive protein, homocysteine, renin, and the urinary albumin-to-creatinin ratio[2]. Other studies focused on CRP levels did not find a direct relation with restenosis[24]. Currently the limitation of existing biomarkers is that even in combination, they only add moderately to the prediction of risk in an individual person[2]. This statement is confirmed by Ware who explains that a risk factor must have a much stronger association with the disease outcome than we ordinarily see in etiologic research if it is to provide a basis for early diagnosis or prediction in individual patients. Most studies are of limited value for the risk stratification of individual patients as we have discovered new biologic variables that lie on the complex pathway leading to chronic disease and death[25]. Therefore the role of experimental research is very important in identifying novel biomarkers since it provides the tools to focus more specifically on the pathophysiological process.

Currently a lot of contradicting data is available of using CRP as a biomarker in cardiovascular disease, a notion merely worsened by the use of different stents types. The approaches thus far may not be specific enough to serve as good reflectors of the pathophysiological process that is initiated by the interventional procedure. CRP is possibly just an indirect reflection of inflammation which is easily become upregulated by other underlying inflammatory processes. The application of individual biomarkers only contributes moderately to risk assessment of individual patients. The prospect of combining multiple known markers could possibly contribute significantly more to the optimization of patient selection and individual tailor-made treatment[1]. The future success of biomarker strategies in this field could possibly depend on the discovery of new biomarkers to complement the current markers and diagnostic strategies. The identification of patients at elevated risk based upon biomarker assessment could be of additive value for the management of patients

receiving stent implantation. In this review, we provide an overview of considered novel potential biomarkers in post-interventional atherosclerotic vascular remodeling with the emphasis on inflammation.

Future biomarkers

Circulating Factors

Several factors involved in the pathophysiological process of post-interventional remodeling may be detected in the circulation soon after the interventional damage, thereby forming potential biomarkers that are easily available such as damage-associated molecular patterns (DAMPs). DAMPs are endogenous structures that can be released upon tissue damage and can be recognised by receptors on inflammatory cells e.g. Toll Like Receptors. Balloon angioplasty with or without stent placement will cause damage to the vessel wall that will cause al release of DAMPs and activate toll-like receptors that cause a release of several inflammatory cytokines and chemokines[26, 27]. Also antibodies may be formed upon (auto-) antigens that become present after the intervention. Original papers on this subject are summarized in the citation overview below.

Citation overview	
Biomarker	**References**
AnnexinA5	28-35
DAMPS	26, 27, 36-62
Cytokines/chemokines	1, 22, 26, 39, 63-85
Plasma antibodies	86-96

Annexin A5

Annexin A5 is a member of the annexin family, a group of highly-conserved Ca2+ dependent proteins that bind to negatively-charged phospholipid surfaces. Annexin A5 is primarily an intracellular protein that is released upon injury and binds specifically and with high affinity to phosphatidylserine (PS)[28]. PS becomes externalized and presented upon the outer cellular membrane during the process of apoptosis, but also during platelet activation and erythrocyte aging[29]. For this reason annexin A5, alone and bound to contrast agents, has been used world-wide for the detection of apoptosis in vitro and in pilot experiments in vivo in patients[28.]

PS serves as an 'eat-me' signal on apoptotic cells for circulating phagocytes. Annexin A5 than binds PS leading to the formation two-dimensional crystals. Annexin A5 thereby may act as a lattice shielding PS from phagocytes and from interacting in phospholipid-dependent coagulation reactions[30, 31]. In addition to its anti-thrombogenic properties, annexin A5 binds with high affinity to oxLDL cholesterol which together with apoptotic cells is present in native atherosclerotic and restenotic lesions in high concentrations[32]. For this reason, annexin A5 is also detectable in high concentrations in (accelerated) atherosclerotic lesions[33].

Next to its presence in the vascular wall, annexin A5 has been suggested in the prevention of pro-inflammatory microparticle formation. Stimulated platelets and

apoptotic cells expressing PS on their membrane have been shown to shed PS-containing membrane-derived microparticles. Annexin A5 is able to inhibit the formation of microparticles by binding to PS on these cells[30].

Annexin A5 is partially removed from the circulation by binding to specific components of atherosclerotic tissues, such as oxLDL and activated or damaged cells. Measurement of plasma annexin A5 concentration requires only limited amounts of venous blood and is therefore an easy-to-perform diagnostic test. Although this information does not allow discrimination between a restenotic and a de novo atherosclerotic lesion, it could certainly be of much additive value to current diagnostic strategies and screening purposes. In addition, it was found that the prognostic value of the oxLDL / annexin A5 ratio is even more sensitive than annexin A5 alone, stressing the importance of combined biomarkers for disease screening[34]. The presence of high concentrations of annexin A5 in atherosclerotic lesions leads to annexin A5 release in the circulation following myocardial infarction[35]. Increased annexin A5 levels are therefore indicative of the extent of myocardial tissue damage. Since annexin A5 levels provide both information on plaque severity in the stable period of atherosclerosis and on infarction severity during acute episodes of plaque rupture, annexin A5 is a highly potential future biomarker of cardiovascular disease progress.

Damage Associated Molecular Patterns (DAMPs)

Restenosis is a late process, although it is believed that events that take place within 72 hours after intervention are already triggering the restenosis process. The intervention will cause severe damage to the vessel wall leading to a release op DAMPs. DAMPs can be seen as endogenous fragments that are recognized by the immune system by toll-like receptors (TLR)[26]. During the whole process of restenosis a continuous process of cell stress, lipid influx, inflammation and matrix degradation, the release of DAMPs will continue. The last decade much focus has been towards the involvement of TLR in cardiovascular disease where the TLRs were predominantly found on circulating cells and in vascular lesions[36-38]. TLRs are membrane-bound receptors located on a variety of immune and non-immune cells including macrophages, endothelium, SMCs and platelets. Release of the DAMPS as endogenous TLR ligands may have serious consequences due to the activation of the TLR signalling pathway on variety of cells carrying TLRs. These cells may than initiate a severe inflammatory response with direct activation of the vessel wall but also platelet activation and infiltration of inflammatory cells[26, 39, 40]. A causal role for TLR4 in post-interventional vascular remodeling was previously demonstrated. Neointima formation, arterial outward remodeling as well as vein graft remodelling were decreased by in TLR4 deficient mice, and TLR4 ligands and TLR4 silencing tools could modify these processes[41-43]. Furthermore TLR4 is importantly involved in the sterile inflammatory response upon CD36 activation by oxLDL particles[44]. Two very important DAMPs that can be linked to multiple TLRs are high mobility group box 1 (HMGB1) and fibronectin-EDA (FN-EDA) that also come available in the circulation upon their release. These DAMPS may potentially serve as ideal biomarkers since they are only upregulated in response to tissue damage, have direct inflammatory effects via multiple TLRs and can also be detected in the plasma[27, 49-51].

Nuclear HMGB1 may become present in the cytoplasm or even outside the cell

where it is known to act as TLR2 and TLR4 ligand[45, 49]. Not only can this release be initiated upon cell stress but also activated macrophages are capable of releasing HMGB1[26, 49-51]. Previously our group was able to detect intra- and extra-nuclear HMGB1 in remodeled vein grafts[42]. Furthermore presence of HMGB1 was detected in atherosclerotic plaques. Although the number of macrophages increased markedly in fatty streaks and fibrofatty lesions, the proportion that expressed HMGB1 did not alter significantly. However, the proportion of macrophages containing HMGB1 in both cytoplasm and nuclei increased markedly[52]. Others showed that elevation of serum HMGB1 level is associated with severe cardiac remodeling complications such as pump failure, cardiac rupture, and eventually in-hospital cardiac death. This was in association with an increased serum C-reactive protein level in these patients. However, in an animal model for myocardial infarction blockade of HMGB1 caused impaired infarct healing and marked scar thinning thereby worsening left ventricular remodelling[53]. Furthermore, HMGB1 serum levels are markedly increased upon surgical thoracic aortic aneurysm repair[54]. HMGB1 is also of interest in other inflammatory disease processes like SLE and kidney ischemia reperfusion[55, 56]. HMGB1 also has pro-thrombogenic features by increasing tissue factor expression on monocytes and inhibiting anti-coagulant protein C pathway in vitro. In vivo the combined administration of thrombin and HMGB1 caused prolonged plasma clotting times[57]. The effect of HMBG1 on platelets via direct TLR4 activation is still unknown. Fibronectin is a part of the extracellular matrix that undergoes severe stress during interventional procedures. Fibronectin-EDA (FN-EDA) is an adhesive glycoprotein spliced from fibronectin and is important in wound healing and can be produced by activated endothelium and fibroblasts. FN-EDA has been implicated in fibroblast differentiation, proliferation and migration and is capable of monocytes activation and induction of inflammation through upregulation of cytokines like interleukin-1α and β and matrix metalloproteinases. Interestingly, FN-EDA is the only spliced variant of FN that binds and activates TLR4[58, 59]. FN-EDA targets antigen to TLR4-expressing cells and induces cytotoxic T cell responses[60]. FN-EDA is also considered to be a TLR2 ligand and therefore has the potential to activate the two most important TLRs in vascular disease. We showed that lack of FN-EDA prevents myocardial remodeling and preserves pump function after infarction[61]. FN-EDA was also found in restenotic lesions with features of accelerated atherosclerosis and in the myocardium in the early phase of the remodeling process following infarction[61]. Additionally absence of FN-EDA reduced atherosclerosis formation. In normal aortas the spliced FN-EDA could not be found, although FN-EDA was found in atherosclerotic plaques and in plasma of atherosclerotic mice. FN-EDA was shown to have effects in both plasma lipoprotein metabolism and in macrophage foam cell formation[59, 62]. Studies with atherosclerotic mice that lack FN-EDA indeed showed that cholesterol levels were lowered[59, 62]. In addition, FN-EDA may influence post-interventional remodeling directly via inducing an inflammatory reaction but also via effects on lipid metabolism.

Cytokines and chemokines

Cytokines and chemokines are important mediators of inflammatory responses and can be easily measured in serum. Both lowered and elevated concentrations of cytokines and chemokines are associated with cardiovascular risk profiles and specifically post-interventional vascular remodeling due to accelerated

atherosclerosis development. Nevertheless their levels can strongly differ due to different pathophysiological processes initiated by different treatment strategies. The treatment strategy (BMS vs. DES) therefore may have strong influence on the reliability of a selected cytokine or chemokine as biomarker[1, 22]. Interestingly conditions after acute myocardial infarction could exacerbate post-angioplasty restenosis by stimulating signaling via TNFα[63]. This may cause differences for patients that undergo scheduled PCI versus patients that had an acute myocardial infarction before PCI. It may therefore be important to look for combinations of specific cytokines besides a selected biomarker. Activation of innate immune response via TLRs will lead to nuclear factor kappa B (NFκB) activation followed by upregulation of cytokines and chemokines[26, 39]. Many different cytokines have been studied in relation to post-interventional remodeling and may be used in combination with specific biomarkers to correlate DAMP presence with remodeling related cytokines. Additionally this may provide better insight in the underlying mechanism of the pathophysiological process and thereby indications for proper treatment strategies. Cytokines could also be interesting to measure the effect of these treatment strategies by checking ratios of pro- and anti-inflammatory cytokines. Here we discuss a few cytokines/chemokines that have been intensively researched in relation to cardiovascular disease and have showed biomarker potential

Tumor necrosis factor α

Tumor necrosis factor (TNF) α is a pro-inflammatory cytokine that is importantly involved in inflammatory responses. Multiple cells including endothelial cells, vascular smooth muscle cells and monocytes-derived macrophages can secrete it. Several studies have shown that blockade of TNF α caused a reduction in neointimal formation via acceleration of endothelium repair, found increased mRNA expression of TNF α in the neointima of damaged vessels which may be upregulated 4000 times compared to resting levels. These kinds of studies also showed a relation with accelerated atherosclerosis[64, 65].

Interestingly, the local delivery of thalidomide as a potent TNF α biosynthesis inhibitor demonstrated a powerful reduction of the neointima formation in mice. In humans single nucleotide polymorphisms in the TNF α gene were found and showed associations with an increased clinical and angiographic risk for restenosis[66]. Angioplasty in peripheral arterial segments gave increased levels of TNF α within 1 hour, although no statistically significant correlation was found between failed angioplasty and the following inflammatory response[67]. Kubica et al stated that the combined analysis of CRP and TNF α might be an effective approach to the clinical restenosis prediction and long-term outcome is markedly influenced by the periprocedural activation of inflammation[68].

Monocyte chemoattractant protein 1

Monocyte chemoattractant protein (MCP)-1 binds to its receptor CC chemokine receptor 2 (CCR2) that belongs to the family of G-coupled receptors and is a chemokine that is capable of attracting immune cells like monocytes. Upon vascular injury MCP-1 recruits monocytes, memory T cells and dendritic cells to the injured site. Attracted monocytes infiltrate the vessel and contribute importantly to neointima formation[69]. MCP-1 is strongly expressed locally in different stages of the remodeling

process. Furthermore, it has strong influence on SMC proliferation. Both mouse models for arterial restenosis as for vein graft remodeling showed a MCP-1 inhibitor showed sufficient reduction in neointima formation. Furthermore studies in which the receptor for MCP-1 was targeted gave similar results[70-72]. No differences in MCP-1 concentrations between patients with acute MI, patients with stable coronary artery disease and healthy individuals were found[73]. However, an inverse correlation was found between MCP-1 concentration at baseline and the time to reperfusion, and a significant decrease in MCP-1 concentration immediately after PCI and lower MCP-1 concentrations over time in patients who developed restenosis within 6 months were found[73]. Elevated baseline level of MCP-1 was associated both with traditional risk factors for atherosclerosis as well as an increased risk for death or myocardial infarction, independent of baseline variables. Interestingly MCP-1 levels are not associated with CRP levels indicating the importance of selecting specific inflammatory markers in stead of general markers like CRP[74]. MCP-1 levels are different amongst patients that received a BMS versus a DES[75]. Furthermore in the same study they found increased monocyte CCR-2 expression 24 hr and 48 hr after stenting in the BMS but not the DES group and changes in plasma MCP-1 after stenting correlated significantly with in-stent lumen loss. Previously another Japanese study already showed a correlation between MCP-1 and the risk for restenosis after stenting[76].

Interleukin 10

Interleukin 10 (IL-10) is one of the most prominent anti-inflammatory cytokines. It may suppress antigen presentation and is capable of inhibiting pro-inflammatory cytokine production. Different animal models for restenosis and atherosclerosis showed protective effects of IL-10 by the use of recombinant human IL-10 or using animals deficient in IL-10.

In humans three polymorphisms significantly increased the risk of restenosis in patients and demonstrate that IL-10 is associated with restenosis[77-79]. IL-10 is however upregulated together with pro-inflammatory cytokines to maintain a balance between pro- and anti-inflammatory cytokines. Most of the time upregulation of pro-inflammatory cytokines exerts the upregulation of IL-10. Peripheral therapeutic angioplasty gave no difference in IL-10 levels compared to patients that underwent only angiography[67]. In a study using BMS after PCI a significant low IL-10 levels was associated with an increase in restenosis after 6 months[80]. The use of undergoing zotarolimus-eluting (zotarolimus is a semi-synthetic derivative of rapamycin that works as immunosupressant) stent implantation combined with pioglitazone significantly reduced neointimal hyperplasia within the stented lesion and attenuated total plaque burden in the in-segment regions of the stent at the 8-month follow-up. These changes were preceded by an elevated IL-10 concentration 10 days after implantation[81].

RANTES/CCL5

CCL5 (RANTES) deposition was involved in wire-induced intimal hyperplasia and blocking of RANTES receptors attenuates neointima formation and macrophage infiltration in animal studies[82]. Two clinical studies focused on the relation of RANTES levels and restenosis. While one of these studies found a decrease of RANTES in

time in the non-restenosis group another found a significant time-dependent increase in the restenosis group[83, 84]. No association was found between RANTES promoter genotype and restenosis[85].

Plasma antibodies
Recent results from murine interventional studies indicate that inflammation and (auto)immune mechanisms are both strong contributors to the development of post-interventional restenosis development have led to the hypothesis that (auto) antibodies are both causally related to restenosis development, and titers could serve as biological biomarkers for the identification of restenosis risk or lesion severity. Longitudinal studies will be required to determine both the diagnostic and predictive values of antibody profiles, but promising candidates have emerged over the past decade, which will be discussed below.
The immune system can be divided into the innate and adaptive systems, which are closely linked and regulated. The innate immunity forms the first line of defense and offers a quick but unspecific response to invading microorganisms, whilst adaptive immunity takes longer to develop, but targets highly specific antigen-bearing foreign intruders. To this end, the former system is comprised of various toll-like receptors, the complement system and cytokines and chemokines, whilst the latter depends on the vast variety of B and T cell receptors and antigen-specific immunoglobulines.

Anti-oxidized LDL and phosphorylcholine antibodies
Immune responses against oxidized forms of cholesterol-containing LDL particles play a critical role in activation and regulation of the inflammatory process that characterizes all stages of atherosclerosisp[86]. LDL cholesterol is the most important risk factor for cardiovascular disease and cholesterol-lowering therapy (statins) alone can reduce CVD-risk by 30-40%. LDL has been found to play a key role in lesion development and LDL oxidation by enzymes such as lipoxygenases primarily occurs in the extracellular matrix in the arterial wall. Oxidative modification of phospholipid fatty acids, degradation of apoB-100 into peptide fragments and modification of these structures by aldehydes derived from oxidized fatty acids leads to the development of immunogenic neo-antigens[87, 88]. These contain pathogen-associated molecular patterns (PAMPs) that are recognized by the pathogen recognition receptors (PRRs) from the immune system, of which TLRs and scavenger receptors are considered to be the most important. TLRs occur both intra- and extracellular and are activated by lipopolysaccharide (LPS) and various other (viral) micro-biological antigens, but also by endogenous ligands such as heat shock proteins and fibronectin extra-domain A[89].Their activation stimulates MyD88-dependent and independent intracellular cascades that eventually all lead to increased NFκB transcription and inflammation.
In both mouse and man, natural anti-oxLDL IgM and IgG antibodies occur, whilst in vitro, antibodies are directed towards malondialdehyde (MDA) and copper-oxidized LDL[87]. These antibodies proved to be exactly similar to those produced by natural occurring T15 B-1 cell clones and all recognize the phosphorylcholine (PC) antigen on oxLDL[90], but also on apoptotic cells and Streptococcus pneumoniae, which share molecular mimicry[91, 92].
These antibodies are suggested to block the oxLDL-uptake by scavenger receptor-bearing macrophages and block foam cell and atherosclerotic lesion formation,

but could also serve as risk markers for atherosclerotic and restenotic lesion development. Several studies have reported increased plasma titers of IgG anti-oxLDL antibodies in patients with angiographically verified coronary artery disease and with acute myocardial infarction[93-95]. Many studies found that low levels of IgM anti-oxLDL antibodies are associated with increased atherosclerosis in hypertensive patients and low levels of IgM anti-PC antibodies with acute myocardial infarction, ischemic stroke and cardiovascular disease in general in both the general population and patients with either hypertension or SLE[93-96]. Therefore, this could also hold true for restenosis due to accelerated atherosclerosis development and the severity of lesion development. In general, these studies have identified anti-oxLDL and specifically anti-PC antibodies as biomarkers for cardiovascular disease monitoring with potentially high additive value to current diagnostic strategies.

Microparticles

Thrombocytes, monocytes and those cellular types lining the arterial wall including endothelial cells and SMCs have been shown to vesiculate and release membrane-shed microparticles in response to cellular activation and apoptosis such as occur during the development of atherosclerotic and restenotic lesions[97, 98]. Membrane integrity is largely controlled by intracellular calcium and caspase homeostasis[99]. Disorganization of the cytoskeleton enables blebbing to occur and disruption of the membrane phospholipid symmetry supports PS externalization[99]. These PS-containing microparticles have been implicated in the development, progression and complications of atherosclerotic lesions and patients suffering from atherosclerotic or restenotic cardiovascular disease display high levels of circulating microparticles and since these microparticles are absent in healthy individuals, their circulating levels prove to allow excellent follow-up of lesion progression and serve as surrogate markers for vascular function[99].

Specifically endothelium-derived microparticles, but not those originating from other cellular types, have been shown to bear high prognostic value in the risk assessment of mortality and major adverse cardiovascular events in patients with coronary artery disease, but also pulmonary hypertension and end-stage renal failure[98].

What can we learn from the lesion itself for selecting novel biomarkers?

In an ideal situation we would like to extract our biomarkers directly out of the lesion since this area previously gave problems and here postinterventional remodeling will start again.

Previously detectable CRP levels in the arterial intima were found preceding the appearance of monocytes. Furthermore, CRP had actually chemotactic capacities by direct influence on monocyte recruitment both in vitro as in vivo[100]. Another study showed that immunoreactivity to CRP was localized to macrophages, SMCs and necrotic areas. Moreover, the immunoreactivity to CRP in coronary atheromatous plaque increases in culprit lesions of unstable angina and it affects restenosis[101]. This may indicate that local CRP levels are much more specific to study while the role of circulating CRP and the relation with post-interventional remodeling may still be difficult to assess since it may be upregulated in multiple ways even independently

of the interventional procedure. Another factor of which its plaque levels were more than 70 times higher in plaques than in plasma is oxLDL in patients undergoing carotid endarterectomy[102]. The same authors also found differences in the oxLDL amount in macrophages rich plaques versus macrophage poor plaques. Interestingly plasma oxLDL levels were only significantly different between control patients and patients with macrophage-rich plaques which may indicate that when studying only oxLDL on the plasma level will not discriminate patients without or patients with a macrophage-poor plaque and may be more helpful in determination of plaque vulnerability than just plaque formation or progression[102]. Bamberg and co-workers showed that different biomarkers of inflammation, vascular remodeling, oxidation, and lipoprotein metabolism maybe associated with different patterns of coronary atherosclerosis as quantified by coronary CT angiography[103]. So lesion phenotype may be very important for the selection of proper novel biomarkers. Only recently a novel study was conducted that uses the knowledge of specific lesions to study the disease process and use it as a predictor of future restenosis and or atherosclerosis occurrence even at other sites than the initial lesion. This Athero-Express study was the first study to provide prospective evidence that plaque composition may predict the risk of restenosis after endarterectomy. Here they found associations for non-vulnerable plaque phenotype to be more prone to develop restenosis[104]. The Athero-Express biobank was also used for a proteomics search approach to identify local biomarkers (selected proteins that have been identified earlier in experimental set-ups with any cardiovascular phenotype but not necessarily with atherosclerosis, osteopontin (OPN) and Macrophage migration inhibitory factor (MIF)) in the atherosclerotic plaque to predict atherosclerotic plaque development in other vascular beds. The authors collected plaques from carotids as well as plaques from femoral arteries and in both cases they found plaque osteopontin (OPN) levels highly predictive for secondary atherosclerotic development. Furthermore, plaque MIF levels were strongly associated with secondary cardiovascular events and showed that the concept not only applies for OPN[105]. Although beyond the scope of this review, the field of proteomic research is evolving and could contribute substantially to the discovery of new biomarkers in post-interventional restenosis and accelerated atherosclerosis.

Most studies are of limited value for the risk stratification of individual patients with the current available biomarkers[25] and are therefore playing a very little role in the prediction of restenosis and decision-making for its exploration and treatment. Furthermore, in many clinical centers it is not possible yet to sample and extract tissue to select for biomarkers patient specifically however combining results of these kind of studies on plaque development and progression together with increased specific knowledge on the complex pathways extracted from experimental research may help us in understanding not only the pathophysiological process but also to select novel biomarkers. They probably will contribute largely to our knowledge and selection of novel biomarkers and may find new correlations between local and circulating levels of biomarkers and post-interventional remodeling and accelerated atherosclerosis. In addition these local studies may even come up with novel biomarkers inside the plaque that can not be detected in plasma due to their low plasma levels, incapacity of being released outside the plaque or just being plaque specific.

Conclusion

Recently published studies have demonstrated that both lowered and elevated concentrations of local arterial and circulating biomarkers are associated with cardiovascular risk profiles and specifically post-interventional vascular remodeling due to accelerated atherosclerosis development. These associations are independent of traditional risk factors and could serve as helpful tools for risk stratification or diagnostic assessment of patients eligible for intensified treatment for clinicians performing target lesion revascularization interventions. Improved assays have identified not only circulating biomarkers, but also cellular-expressed receptors, co-factors and microparticles that all directly causally involved in disease progress, but also indirectly as biomarkers of inflammatory status and vascular function. Provided these findings are replicated in other studies, the combined power of current diagnostic strategies with the latest tools and multiple biological risk markers could contribute significantly to the optimization of patient selection and future individual tailor-made treatment.

Executive summary

Introduction
- Coronary heart disease remains the leading cause of death and is caused by atherosclerosis, a chronic inflammatory disease

Underlying causes of restenosis
- Post-PCI restenosis is determined by biological, arterial, stent and technical factors

Inflammation status as biomarker
- Biomarkers can be divided into local, circulating and circulating cell-bound markers and are most valuable when causally related to disease progression

Circulating biomarkers
- Circulating markers and ligands of the innate immune system such as fibronectin, inflammatory cytokines, annexin A5 and natural antibodies towards oxidized LDL cholesterol and HMGB-1 can be highly predictive
- Inflammatory receptors on circulating leukocytes such as TLRs and scavenger receptors, but also microparticles are directly implicated in and strongly indicative of atherosclerotic vascular remodeling

What can we learn from the lesion itself for selecting novel biomarkers?
- Biobanks containing mRNA and protein profiles from numerous atherosclerotic plaques are highly valuable for local biomarker screening

Conclusion
- Biomarkers of inflammation status possess the highest predictive value for accelerated atherosclerosis disease progression, especially when combined and in addition to current diagnostic strategies

Future perspective

The insight into the development of atherosclerosis and post-PCI restenosis has developed very quickly over the past decade. Ever since, atherosclerosis is primarily

viewed as a chronic inflammatory disease due to a dysfunctional immune response towards the arterial wall. To this end, the focus on atherosclerotic biomarkers has shifted from traditional markers that serve as risk factors, such as hypercholesterolemia, towards markers of systemic inflammation (e.g. C-reactive protein) and arterial dysfunction. The important notion remains that causal factors are additionally powerful predictors of disease progression and the same would apply for inflammatory markers. The field of diagnostic and treatment-evaluation markers will shift in the same direction, guided by new insights, and rely heavily on the additional value of new biomarkers to the current diagnostic strategies. Epidemiologic assessment of additional value from combining biomarkers is a powerful tool to discover new biomarkers entities for the development of highly specific assays, specifically for genetic biomarkers such as polymorphisms that are associated with disease risk.

The field of biomarkers for accelerated atherosclerosis and post-interventional vascular remodeling has changed due to the introduction of drug-eluting stents. These stents have rendered various markers of little use, since they closely followed the inflammatory reaction towards BMS placement and are currently prevented by adequate local drug release. Their usefulness could be further compromised in future due to the ever-increasing application of drug-eluting balloons. Nevertheless, new inflammatory factors such as intraplaque and circulating proteins, natural antibodies, microparticles and cellular receptor expression could prove to be of highly-specific and diagnostic value. Furthermore, application of such screening assays would allow for optimal treatment evaluation such as occurred in the past with the introduction of cholesterol-lowering statin therapy.

Development and application of future biomarkers requires clinical validation, which remains a time-consuming and expansive entity, and this uncertainty is inherently (most notably on safety issues) present at the final stages of drug validation, limiting future biomarker development. In spite of this, investigations continue to proceed and will improve diagnostic and treatment accuracy of post-interventional atherosclerotic vascular remodeling.

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