

The vulnerable patient: Refocusing on the plaque?

Wouter J. Eijgelaar; Sylvia Heeneman; Mat J. A. P. Daemen

Experimental Vascular Pathology (EVP) Division, Department of Pathology, Cardiovascular Research Institute Maastricht (CARIM), University of Maastricht, Maastricht, The Netherlands

Summary

The term 'vulnerable plaque' is used to refer to the lesions that are prone to rupture and may cause life-threatening events like acute coronary syndrome or stroke. The study of the vulnerable plaque phenotype and its detection has attracted increasing interest over the past decades. During this time, there have been some remarkable transitions in the paradigm on methods to identify patients at risk or patients to treat. Whereas formerly, the key factors used to determine an individual's risk were primarily population-based traditional risk factors such as age, sex, body mass index, hypertension etc., new approaches are based on conditional risk factors that represent an individual's current risk of suffering a cardiovascular event. These population based risk factors fall short in predicting near-future events in a high-

risk individual. In the early 2000s, the focus of research into surrogate markers for cardiovascular event prediction shifted from the vulnerable plaque to the identification of the vulnerable patient. This new paradigm stimulated a number of new initiatives that aimed to identify vulnerable patients by testing systemic biomarkers that could identify patients at high risk for cardiovascular events. A second research paradigm is refocusing on the plaque by searching for plaque-derived biomarkers and non-invasive imaging modalities to assess characteristics of a plaque that determine its vulnerability. Although both concepts are attractive, they still need proper validation in large multicenter cohorts, while cost-effectiveness arguments also need to be assessed.

Keywords

Cardiovascular event prediction, imaging, risk factors, biomarkers, atherosclerosis

Thromb Haemost 2009; 102: 231–239

Introduction

Atherosclerosis is a progressive disease that affects nearly all individuals in the Western world. After its onset during childhood, it slowly progresses from initial intimal thickening toward more advanced lesions in early and late adulthood. Such advanced lesions can remain stable or develop into plaques that are prone to rupture, so-called vulnerable plaques. Rupture of these vulnerable plaques is thought to cause life-threatening events like acute coronary syndrome (ACS) or stroke. In fact, coronary plaque rupture is responsible for approximately 75% of coronary thrombi that lead to myocardial infarction and/or death (1–4). The clinical events caused by progressive atherosclerosis currently remain the primary cause of death in Western society.

In the US, cardiovascular disease caused one of every 2.9 deaths in 2006. An estimated 785,000 primary coronary events and 470,000 recurrent events are expected to occur in the US in 2009, and it is estimated that an additional 195,000 silent myo-

cardial infarctions occur each year. Numbers of stroke are comparable, with 795,000 events, including 610,000 primary and 185,000 recurrent events. Although death rates have decreased by approximately 30% over the 1995–2005 period, the estimated numbers of people who suffer a cardiovascular event (event rate) has gradually increased over the last two years (5, 6). These high incidence figures clearly indicate the need for better and earlier diagnostic modalities to reduce the numbers of cardiovascular events. Early detection of patients at risk is essential to allow adequate prevention of such events. Although traditional risk factors for cardiovascular disease have been defined, they fall short when it comes to predicting the personal risk of plaque rupture-induced thrombosis in groups that they classify as being at intermediate or high risk.

The study of the vulnerable plaque phenotype and its detection has attracted considerable interest in recent decades. During this time, there has been a remarkable shift in the paradigm on methods to identify patients at risk or patients who need to be

Correspondence to:
Wouter J. Eijgelaar, MSc
Dept. of Pathology, Cardiovascular Research Institute Maastricht (CARIM)
University of Maastricht
P. Debyeplein 25, 6229 HX, Maastricht, The Netherlands
Tel.: +31 433876626, Fax: +31 433876613
E-mail: Wouter.Eijgelaar@PATH.unimaas.nl

Received: January 13, 2009
Accepted after major revision: June 1, 2009

Prepublished online: July 3, 2009
doi:10.1160/TH09-01-0030

treated. Whereas formerly, key factors used to determine an individual's risk were primarily population-based traditional risk factors, new approaches are based on conditional risk factors, which vary with the current state of the vessel wall and atherosclerotic plaque and thereby represent the current probability that an individual will suffer a cardiovascular event. This new approach was introduced in 2003 by Naghavi et al. in a review series called 'From vulnerable plaque to vulnerable patient' (2, 3, 7).

The vulnerable patient paradigm is nowadays widely supported in the atherosclerosis field (8, 9) and is based on the general concept that atherosclerosis is a systemic disease. It moves the assessment of the degree of atherosclerosis in a specific artery to the assessment of a patient's vulnerability to life-threatening clinical events. Combined with the wide acceptance of atherosclerosis as a chronic inflammatory disease (10–12), this concept has led to a series of new approaches to risk assessment of cardiovascular events. These approaches mostly focus on detection of serum levels of markers of inflammation, blood thrombogenicity and matrix degrading capacity that correlate with coronary artery disease (13), while some new initiatives pursue the use of biomarkers on the circulating cells such as white blood cells and blood platelets that are suitable for discriminating patients with an increased cardiovascular risk.

Another approach to risk stratification of high-risk patients refocuses on the plaque. It uses the characteristics of the atherosclerotic plaque and our expanding knowledge on the biology of plaque progression as a base for developing novel circulating and imaging biomarkers.

Advances in the field of circulating biomarkers might lead to better and more individualised cardiovascular risk prediction and non-invasive plaque imaging modalities might be able to assess the vulnerability of a particular plaque at a particular point in time. However, the predictive value of both approaches has not been proven to date. Both concepts may not only be helpful in improving cardiovascular risk prediction, but also in the development of a more personalised treatment. They may speed up clinical trials by providing new surrogate endpoints that will allow smaller sample sizes and shorter trial durations compared to trials using morbidity and mortality as endpoints.

The aim of our review is to call the reader's attention to these two novel and possibly complementary concepts in cardiovascular risk prediction.

From population-based risk factors to individualised risk stratification

Over the past 60 years, a number of 'traditional' cardiovascular risk factors have been defined, including age, sex, body mass index, hypertension, diabetes, smoking, lipid profile and family history of premature coronary artery disease. Most of these were derived from the longitudinal Framingham heart study, which has been monitoring a large number of individuals since 1948. Having started off with 5,209 adult subjects from the small US town of Framingham, the study is now in its third generation of participants (14). A second large initiative that led to the identification of risk factors for coronary heart disease is the Prospec-

tive Cardiovascular Munster (PROCAM) study. Recruitment for this study took place between 1979 and 1991, during which time 23,616 individuals were enrolled (15). Most of the currently used traditional risk factors are derived from studies such as the Framingham and PROCAM studies. However, a study of the performance of the National Cholesterol Education Panel III Guidelines, commonly used for the stratification of patients for treatment in the US, indicated that the current risk stratification tools fall short when it comes to the prediction of acute myocardial infarction (AMI). It was shown that a staggering 75% of patients who had recently experienced an AMI did not qualify for medical treatment according to these guidelines (16). Many tools have been developed to increase the sensitivity and specificity of these risk factors, such as the Framingham risk equations/algorithms and the PROCAM score, which deliver simple point-scoring schemes for calculating the risk of an acute coronary event by combining and grading the individual factors (15, 17–19).

All of the above risk factors reflect an individual's predisposition to develop severe atherosclerosis, due to either genetic or behavioural factors. The result is a population-based individual risk of developing an acute coronary event within 10 years, but it does not represent the plaque load or risk of plaque rupture for an individual patient (or an individualised plaque) and fails to identify near-future victims of a cardiovascular event. Therefore, a novel approach that focuses on surrogate markers for cardiovascular event prediction and the identification of the vulnerable patient was introduced.

The vulnerable patient

The term 'vulnerable patient' was introduced in a review series in the early 2000s, which defined the characteristics of a vulnerable plaque that is susceptible to rupture and therefore to clinical complications. Active inflammation, a thin fibrous cap with a large lipid core, endothelial denudation with superficial platelet aggregation, a fissured plaque and severe stenosis were determined as major characteristics, while superficial calcified nodules, yellow colour on angiography, intraplaque haemorrhage, endothelial dysfunction and expansive outward remodelling were determined to be minor identifying characteristics of a vulnerable plaque (2, 3).

Following the vulnerable plaque paradigm, many studies to identify vulnerable plaques in patients showed that lesions with evident atherothrombosis were not necessarily the ones with the highest degree of major vulnerable plaque characteristics. This finding, along with the fact that multiple vulnerable plaques were identified in individual patients and that asymptomatic patients had plaques that met the major criteria for a vulnerable plaque, signalled the end of the vulnerable plaque paradigm and caused a shift in focus, away from the identification of individual vulnerable plaques towards the identification of vulnerable vasculature and ultimately vulnerable patients. The vulnerability of such a vulnerable patient is not only dependent on the vulnerability of the plaques, but also on factors like the thrombogenicity of the blood and the electric stability of the myocardium (Fig. 1) (20–24).

This novel paradigm has stimulated a number of new initiatives that aimed to identify vulnerable patients at high risk for

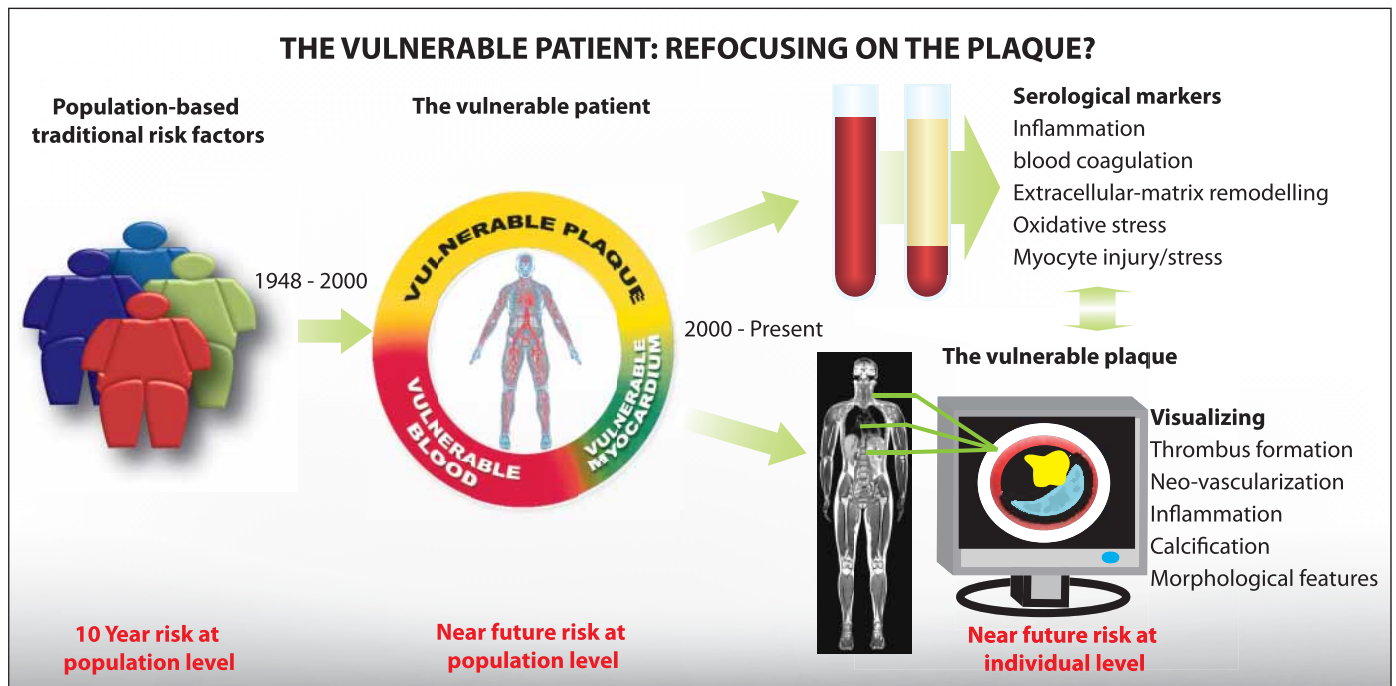


Figure 1: The vulnerable patient: Refocusing on the plaque?
Adapted from Naghavi (Vulnerable patient) (3). Over the years, several approaches to cardiovascular risk prediction have been pursued. Initially the approaches focussed on the vulnerable plaque. Population-based studies such as PROCAM and Framingham in this period led to the identification of risk factors termed 'traditional risk factors', which indicate a person's predisposition to develop severe atherosclerosis. The paradigm shift that described atherosclerosis as a multifactorial systemic disease instead of a disease occurring in one specific vessel, led to a shift in focus to the vulnerable patient. The vulnerable patient paradigm

stimulated a number of new initiatives that aimed to identify vulnerable patients by testing conditional biomarkers that are not only dependent on the vulnerability of the plaques, but also on factors like thrombogenicity of the blood and the electric stability of the myocardium. This has led to a large number of novel surrogate markers of the risk of a cardiovascular event. Another approach that receives increasing interest, refocuses on the plaque through non-invasive imaging modalities and plaque-derived biomarkers that assess characteristics of the plaque which determine plaque vulnerability.

cardiovascular events by testing for systemic biomarkers. Current applications of these systemic biomarkers mostly involve the detection of serum levels of markers of inflammation, blood thrombogenicity and matrix degrading capacity that correlate with coronary artery disease (13). These markers are used to predict a patient's vulnerability to near-future cardiovascular events.

One of the most established serum markers is C-reactive protein (CRP). The introduction of highly sensitive immunoassays for CRP (High Sensitive CRP [hs-CRP]) in the mid 1990s led to the idea that increased values of hs-CRP in a range that was previously considered to represent normal baseline levels, predict future events (25–27). CRP is an acute phase protein with serum concentrations that increase during systemic inflammation. It is produced by hepatocytes, and serum levels are rapidly raised up to a factor of 10,000 by an inflammatory stimulus. The protein has very stable baseline serum concentrations, which are characteristic for each individual. By 2008, over 40 studies had investigated the significance of serum CRP levels for the prediction of future events, such as cardiac ischaemia, stroke and peripheral artery disease (28–34).

Since CRP is an acute phase protein that responds to inflammation and tissue damage, it has low positive predictive value and thus high false positive rates in predicting future cardiovascular events. A meta-analysis of 22 population-based prospective studies, with a mean follow-up of 12 years and 7,068 cardio-

vascular events, (35) found that the odds ratio for cardiovascular events after correction for several other established risk factors was 1.6. CRP thus only marginally adds to the predictive value of established risk factors for coronary heart disease.

Besides CRP, studies have also assessed the predictive value of serum levels of other inflammatory markers, such as IL-6, IL-3, IL-8, M-CSF and soluble CD40 ligand (33, 36–40). Serum levels of IL-6 and M-CSF are linked to CRP, as M-CSF-induced activation of monocytes and macrophages leads to increased IL-6 production by these cells, and IL-6 in turn induces CRP production in hepatocytes (41). The odds ratios are similar in magnitude to that reported for CRP (35).

The main drawback of all of these serum markers is a low positive predictive value. Although all show a strong correlation with cardiovascular disease in a relatively healthy population, the inverse claim that elevated levels of these inflammatory markers indicate a high risk of cardiovascular events is difficult to substantiate, due to their induction by other inflammatory stimuli besides atherosclerosis. Nevertheless, inflammatory markers can provide predictive value independent of the classical risk factors in specific subpopulations, as has been shown for IL-6 in ST-segment elevation myocardial infarction (STEMI) patients and patients undergoing coronary angiography, as well as in the Veterans Affairs Diabetes trial (38, 42, 43). One way to increase the positive predictive value of the inflammatory markers

is to include not one but a large number of markers and combine these with the traditional risk factors. However, the addition of novel biomarkers to the traditional risk factors resulted in only small increases in the ability to classify risk. This was shown in a study by Wang et al. in which the performance of this multi-marker approach was tested on 3,209 of the Framingham heart study participants (44). Assessment of a combination of 10 biomarkers with a follow-up of 7.4 years showed a hazard ratio of only 1.84 and an insignificant increase in the ability to classify risk, as indicated by a C statistic of 0.76 to 0.77.

In addition to the inflammatory markers, many novel serum markers that correlate with the risk of cardiovascular events have been identified and are currently being tested. These markers are mostly involved in processes that lead to cardiovascular events, such as blood coagulation, extracellular-matrix remodelling, oxidative stress and myocyte injury/stress. Two recently published elaborate reviews assessed several of these novel markers, such as fibrinogen, myeloid-related protein 8/14, adiponectin, brain natriuretic peptide (BNP) and matrix metalloproteinase-9 (MMP-9), for their ability to predict cardiovascular events. The reviews concluded that no additive value to the Framingham risk score had been proven for any of these novel markers. Moreover, the lack of commercial assays for a number of these markers limits the adequate assessment of standardisation and accuracy for clinical use (13, 45).

Much effort is still being invested in the identification and implementation of novel biomarkers for atherosclerotic risk prediction. Despite the somewhat disappointing results of the biomarkers described above, much is expected of biomarker panels in which relative changes in biomarkers indicate the risk of a cardiovascular event.

Alongside research into the markers described above, large studies are currently evaluating new approaches to biomarker research and the identification of vulnerable patients. Two prominent programmes that are based on the vulnerable patient paradigm are the High Risk Plaque (HRP) initiative, chaired by Valentin Fuster and Erling Falk (<http://www.hrpinitiative.com/>), and the Dutch Circulating Cell project, which is funded by the Center for Translational Molecular Medicine (CTMM) (<http://www.ctmm.nl>) (46).

The HRP initiative aims to develop and validate imaging modalities and biomarkers for the early detection of individuals with vulnerable plaques, in a very large prospective observational study involving 7,300 Framingham medium-risk volunteers, men aged 55–80 and women aged 60–80, who do not have clinical symptoms of cardiovascular disease or other medical conditions that can interfere with the study. Many coronary heart disease-related parameters will be assessed at baseline, involving a series of physical measurements, an ECG and blood tests, as well as a stepwise approach involving several non-invasive imaging modalities of the relevant vascular beds (peripheral, carotid and coronary arteries). This stepwise approach will start with low-resolution imaging modalities such as ultrasound (intimal media thickness), computed tomography (CT) (coronary artery calcium score) and ankle brachial score among 6,000 patients, using the results of these modalities as criteria to select approximately 1,000 patients who will undergo higher-resolution imaging modalities, such as magnetic resonance imaging (MRI) and cardiac CT angiograms

(CCTA), to better characterise their atherosclerotic disease. A three-year follow-up period will be used to determine whether any of the baseline methods and tests could identify individuals at higher risk of coronary artery disease. Three biomarker discovery studies run in parallel with the HRP imaging study, and the results of these studies will be validated in the patients enrolled in the imaging project. While the HRP initiative is expected to reveal which image modality will be able to detect patients at risk, it does not aim to define plaques at risk.

The Circulating Cell project (see www.ctmm.nl) explores the value of the pool of circulating cells as biomarkers for progression of atherosclerotic disease. It is an example of a programme based on the vulnerable patient paradigm that aims to develop novel tools to assist in the prediction of cardiovascular events. Instead of the widely applied search for serum markers, this programme will target the most active components of the blood, the circulating cells that interact with the vessel wall and can be considered messengers of disease. Multiple approaches are used to investigate the extent to which individual blood components represent the current state of the vascular tree. These blood components include serum protein/marker levels, platelet function and proteomics, platelet/monocyte interaction and function, transcriptomics, proteomics and the distribution and activation of circulating white blood cell subsets. Correlations between circulating cell gene expression levels and the state of atherosclerosis have been shown in studies comparing leukocyte mRNA levels of patients with severe coronary artery disease with those of patients without angiographic signs of coronary artery disease, or studies linking the risk of in-stent restenosis to the transcriptome of circulating leukocytes (47, 48). Patino et al. found that the correlation between the transcriptome of circulating monocytes and plaque-residing macrophages of patients undergoing carotid endarterectomy was stronger than the correlation between the transcriptome of lung macrophages and monocytes from healthy subjects. Thorough investigation of the active mechanisms involved in plaque destabilisation and thrombus formation in a large cohort of patients may lead to the identification of surrogate markers that predict near-future events more accurately than the currently available markers (49).

New programmes, such as the multi-marker approach of Circulating Cells and the HRP initiative, have the potential to improve risk stratification based on the vulnerable patient paradigm.

From vulnerable patient refocusing on the plaque

In parallel with the search for systemic biomarkers, another paradigm refocusing on the plaque is emerging. The currently accepted vulnerable plaque characteristics are mostly based on cross-sectional or retrospective studies relating the characteristics and morphological features of ruptured plaques to the vulnerability of a plaque. Since the tissues are solely available through autopsy or, in the case of for example carotid endarterectomy, samples from symptomatic patients, there have not been any prospective studies following asymptomatic plaques through time until rupture.

The value of approaches based on biomarkers inside the plaque that reflect its vulnerability is currently being investigated. Such an approach is pursued in one part of the HRP initiative, the Plaque Biology Study. This study was performed at our lab and involved collecting surgical specimens of carotid atherosclerotic plaque, obtained from 25 patients who underwent carotid endarterectomy, containing both a stable and a ruptured plaque phenotype within a single surgical specimen. The differences in composition and biological processes between the ruptured and stable plaque areas are being studied using immunohistochemistry, proteomics, metabolomics, lipidomics and transcriptomics. In the end, combining all this information is expected to provide a thorough understanding of the processes related to plaque rupture, and allow plaque-derived biomarkers to be selected (<http://www.hrpinitiative.com/hrpinit/research/plaque-biology.jsp>).

In addition, Hurks et al. are investigating the predictive value of local plaque composition for systemic cardiovascular outcome. Initial results from their study using the longitudinal Athero-express biobank indicated that the local plaque characteristics have strong predictive value for cardiovascular events elsewhere in the vascular tree (50).

We have used atherosclerotic plaques as a source for the identification of serological markers for ruptured peripheral plaques. Using symptomatic plaques that were surgically removed from the femoral, iliac, popliteal and carotid arteries, a phage-display library containing cDNAs preferentially expressed in ruptured peripheral human atherosclerotic plaques was obtained. Serological antigen selection to identify cDNA products that specifically interact with antibodies in sera of patients with proven ruptured peripheral plaques resulted in two cDNA products, E1 and E12, which were able to discriminate between patients with ruptured peripheral lesions and patients with stable peripheral lesions with 100% specificity and 76% sensitivity. Ninety-three percent of the acute myocardial infarction patients in the study tested positive for the selected markers, whereas none of the stable angina pectoris patients tested positive (51). These results indicate the potential of plaque-derived biomarkers and their translation into serological tests as part of the development of tools for atherosclerotic risk prediction. Longitudinal prospective studies are needed to validate the added value of these markers for atherosclerotic risk prediction.

Molecular profiling of plaque material, in addition to being valuable in unravelling ongoing processes in the plaque, may also lead to specific markers that predict rupture. These can be assessed serologically but also through molecular imaging techniques (52).

The rapid development of imaging devices and modalities has led to a shift in the use of these tools in the clinical setting. Every year sees the introduction of new applications and improvements in terms of resolution, contrast and detection limits. At the same time, the number of targets and contrast agents for molecular imaging is growing rapidly, with new modalities being introduced to assess and quantify plaque vulnerability.

The predictive value of non-invasive procedures, such as electron-beam CT (EBCT), multidetector row CT (MDCT), positron-emission tomography (PET), carotid artery ultrasound, carotid/cardiovascular MRI and coronary CT, which assess the

pathology of the vessel wall and aim to identify rupture-prone plaques, is currently being tested.

This is underlined by the first SHAPE (Screening for Heart Attack Prevention and Education) guideline, published by Naghavi et al. in 2006. They describe the observation that most heart attacks and strokes occur in people who are not classified as high risk by the traditional risk factor-based approach recommended in the United States (Framingham Risk Score) and therefore propose non-invasive screening of all asymptomatic men 45 to 75 years old and asymptomatic women 55 to 75 years old (except those defined as very low risk). They propose to subsequently treat individuals with subclinical atherosclerosis that are detected using coronary artery calcium scoring determined by CT and carotid intima media thickness and plaque measured with ultrasound imaging (53).

Ultrasound imaging to determine the degree of stenosis in carotid arteries of symptomatic patients is a well-established technique used in clinical settings (54). Although this is a suitable technique to stratify symptomatic patients for carotid endarterectomy, a stratification based on the percentage of stenosis has very poor predictive value in asymptomatic patients. It is known that 70% of plaque ruptures occur in lesions with less than 50% stenosis (55, 56). As the risk of stroke associated with asymptomatic carotid stenosis is only 6% within three years (57, 58), there is a clear need for more specific imaging modalities that can identify asymptomatic patients with less than 70% carotid artery stenosis who are nevertheless at high risk for cardiovascular events.

EBCT is able to identify and quantify calcium content in coronary arteries. Arterial calcification, however, has no proven correlation with plaque vulnerability. Newer modalities such as MSCT or MDCT provide more details of plaque morphology (59, 60), and are able to distinguish fibrous-rich from lipid-rich plaques (61). A recent study showed a lower density of the content of the plaque in unstable angina pectoris patients compared to stable angina patients. In addition, MDCT has been shown to be able to identify the presence and severity of obstructive coronary artery disease and subsequent neovascularization in symptomatic patients (62, 63). MDCT provides very high sensitivity and specificity, 93–95% and 85–90% respectively, when validated by invasive coronary angiography (CAG) for the detection of significant stenosis in culprit and non-culprit vessels (64–66). CAG has been the gold standard for the diagnosis of coronary artery disease in recent decades, and new non-invasive imaging modalities are therefore often compared with CAG to determine their sensitivity and specificity. The validity of CAG is questionable, since CAG only assesses the lumen and the degree of stenosis, which have very low predictive value, as described above. Ideally, new non-invasive imaging modalities have to be validated against histological analysis, and if histological samples are unavailable, against invasive high resolution techniques such as intravascular ultrasound.

MDCT not only has a relatively low resolution compared to techniques like MRI, it is also regarded as a moderate- to high-radiation diagnostic technique, with radiation levels in the range that is reported to cause cancer, although the exact risk increase is still a matter of debate (67). The level of radiation exposure is directly linked to the image quality and penetration depth, so

image quality needs to be balanced against the risk of fatal cancer.

MRI is one of the most informative non-invasive plaque imaging modalities. It not only allows characterisation of the fibrous cap, calcification and lipid core of the plaque, but also provides information on cellular plaque components through contrast-enhanced molecular imaging (68). In addition, it provides important information on the surrounding tissue, such as the myocardium and/or the brain, in one single session. This information on possible end-organ damage is a major advantage in symptomatic patients, but also indicative in asymptomatic event-free survival patients in whom signs of dysfunctional myocardium predict the occurrence of adverse cardiac events or death (69).

One clear example of an application of molecular imaging of plaque vulnerability is the visualisation of plaque macrophages involved in plaque progression. This is achieved through the use of Ultrasmall Superparamagnetic Iron Oxide particles (USPIOs), which are phagocytised after intravenous injection by plaque-residing macrophages and can be detected on MRI as a signal void (70, 71). This reveals the degree of inflammation of a plaque and thus potentially provides greater predictive value than the traditional risk factors. This method has, however, not yet been approved by the FDA and is based on a negative MRI signal, which is hard to quantify, hampering clinical decisions. Although MRI of the plaque is a very promising modality that could provide large amounts of information on plaque vulnerability, its clinical application is still in a very early stage, and its true predictive value has not been shown in large multicenter trials yet.

Nuclear techniques, such as PET, potentially provide higher detection sensitivity to nanoparticles than MRI, as it allows detection in the range of picogram concentrations of nanoparticles instead of the microgram-range concentrations that can be detected by MRI. Combined with the anatomic information provided by CT, PET-CT forms a highly sensitive imaging modality for nanoparticle-guided atherosclerotic plaque characterisation (72, 73). One of the major drawbacks of PET-CT is its relatively low resolution, which renders imaging of small vessels such as coronary arteries challenging. ^{18}F -Fluoro-2-DeoxyGlucose (^{18}F -FDG) is a positron emitter that is used in oncology to detect malignant cells and has recently been introduced to detect atherosclerotic plaques. ^{18}F -FDG competes with the uptake of glucose into the cell by a membrane carrier transport mediated mechanism, generally through glucose transporter 1 (GLUT-1). ^{18}F -FDG accumulates more quickly in tumour cells compared to non-neoplastic cells (74, 75). The same effect occurs in plaque macrophages, allowing the non-invasive detection of highly inflamed and thus more vulnerable plaques (76, 77).

Successful introduction of risk assessment of plaque rupture through non-invasive imaging is still hampered by several factors. 1) A major drawback of the fast pace of development of imaging modalities is the lack of adequate clinical validation. New modalities or improvements of existing techniques are outrunning the clinical validation of older techniques, rendering clinical validation of current imaging modalities unattractive. Once, or even before, a novel technique has been properly validated, it is already outdated. 2) The current techniques only assess morphological parameters, while neglecting other measurable pa-

rameters, such as biomechanical, biological and molecular changes in rupture-prone plaques. 3) Previous initiatives validating imaging modalities mostly focused on one or two techniques, and did not integrate the results of multiple modalities with other known risk factors, nor did they validate the imaging results by one-to-one comparisons with a detailed histological analysis of the excised plaque. 4) There are currently no serial studies that provide surrogate imaging markers for the risk of a cardiovascular event. That is, no measures that change significantly prior to rupture and thus are predictive of an imminent event are currently known. An example of the attempts to assess plaque features non-invasively are the studies of Yuan et al. in which the capabilities of MRI for the assessment and quantification of carotid plaque composition are tested (78–83).

Studies that measure traditional risk factors together with systemic biomarkers and plaque-specific features derived from non-invasive multi-modality imaging will eventually lead to a highly sensitive and specific near-future risk prediction model for individual high-risk patients.

Visualising the vulnerability of plaques

Assessing the vulnerability of a plaque through non-invasive imaging requires the introduction of new plaque parameters that are linked to vulnerability in an imaging-based risk prediction algorithm. Narula et al. recently published an elaborate review on the subject of imaging vulnerable plaques in asymptomatic patients. They presented an overview of characteristics of vulnerable plaques whose predictive value has been explored in both invasive and non-invasive imaging modalities (52).

Imaging thrombus formation through targeted MRI or PET will help to identify non-stenotic ruptured lesions in asymptomatic patients. As thrombosis is one of the ways a plaque progresses (84), active thrombosis within a plaque will increase the risk of stenosis and the subsequent clinical symptoms, and can thus be a surrogate for cardiovascular event prediction. In addition, blood thrombogenicity determines the extent to which the blood reacts to vascular changes such as erosion and rupture of the plaque (4).

Several studies have explored non-invasive imaging of thrombus formation (85, 86). EPIX pharmaceuticals developed a fibrin-specific gadolinium-based MRI contrast agent, EP-2104R, which has entered a phase II clinical trial. Although initial results indicate that EP-2104R allows for selective molecular MR imaging of thrombi in the arterial vasculature and the heart in humans (87, 88), the programme has been discontinued by the company. Von zur Muhlen et al. visualised thrombus formation by labelling activated platelets with a contrast agent consisting of microparticles of iron oxide (MPIO) and a single-chain antibody targeting ligand-induced binding sites (LIBS) on activated glycoprotein IIb/IIIa. They showed that the contrast agent bound to the luminal surface of lesions in symptomatic patients. Moreover, thrombolytic treatment decreased signal enhancement, validating the specificity of the contrast agent. This study suggests the potential application of imaging of thrombus formation in the detection of vulnerable plaques in asymptomatic patients (89).

Plaque vulnerability is also thought to be affected by neovascularisation. It has been hypothesised that the growth of new

intraplaque microvessels promotes plaque progression, mainly due to the leaky nature of these neo-vessels, possibly through defects in endothelial integrity (90, 91). Erythrocytes contain relatively large amounts of cholesterol and the pro-oxidant haemoglobin/Fe in their cell membrane, and leak into the plaque area. They thereby contribute to intra-plaque free cholesterol and reactive oxygen species (ROS) and hence to lipid-rich necrotic core formation and plaque destabilisation. In addition, leukocyte infiltration through these vessels stimulates plaque progression (92). Several studies have examined non-invasive imaging of the microvessels through $\alpha_v\beta_3$ integrin-targeted gadolinium, using MRI and using gadolinium-enhanced dynamic contrast MRI. Detection of intraplaque microvessels will help to determine the role of these neo-vessels in plaque vulnerability and their relevance in event prediction (93–95).

There are thus several new imaging tools using biological information about the plaque at risk in different stages of development, but the application of such non-invasive imaging modalities in large-scale screening of asymptomatic high-risk patients is not yet feasible. Their additional value in near-future risk prediction needs to be properly validated in large multicenter trials. Another neglected point is the costs involved in large-scale high-tech screening of asymptomatic patients. Current costs do not allow large-scale screening and it is most likely that these modalities will initially only be applied in very high-risk patients, as determined by traditional risk factors. If individualized treatment tools become available, the added value of more detailed screening will be high. These modalities will therefore most likely become more common in cardiovascular risk assessment.

Conclusion

In recent years several approaches to cardiovascular risk assessment have been pursued. The paradigm shift that described

atherosclerosis as a multifactorial systemic disease instead of a disease occurring in one specific vessel, has led to the development of several new approaches to improve the risk assessment of cardiovascular events. The first included large population-based studies that defined factors determining a predisposition to suffering a cardiovascular event within the next 10 years and yielded the so called traditional risk factors. Subsequently it became apparent that a more individualised risk prediction was needed. The vulnerable patient paradigm, has led to numerous studies investigating the predictive value of new factors that are related to the biological systems involved in cardiovascular events and may yield more individualised biomarkers. In parallel with the ongoing efforts to identify and validate new systemic risk factors, there is increasing interest in another approach that refocuses on the plaque through non-invasive imaging modalities and plaque-derived biomarkers. The feasibility and predictive value of many new imaging techniques as well as surrogate imaging markers for the risk of a cardiovascular event are currently being explored. These techniques still need proper validation in large multicenter cohorts, and cost-effectiveness arguments need to come into play as well.

One future approach to cardiovascular risk prediction and primary prevention, will consist of initial screening based on traditional markers and novel serum, circulating cell and plaque based biomarkers, followed by cheap and relative low resolution non-invasive imaging to assess the presence and volume of a plaque. Follow-up with high-resolution imaging, to provide measurements of the plaque characteristics, can then be used to pinpoint the patient to treat. Early detection of the patient to treat will allow clinical interventions to prevent a cardiovascular event.

References

- Falk E. Pathogenesis of atherosclerosis. *J Am Coll Cardiol* 2006; 47: C7–12.
- Naghavi M, Libby P, Falk E, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part II. *Circulation* 2003; 108: 1772–1778.
- Naghavi M, Libby P, Falk E, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part I. *Circulation* 2003; 108: 1664–1672.
- Thim T, Hagensen MK, Bentzon JF, et al. From vulnerable plaque to atherothrombosis. *J Intern Med* 2008; 263: 506–516.
- Rosamond W, Flegal K, Furie K, et al. Heart disease and stroke statistics--2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 2008; 117: e25–146.
- Lloyd-Jones D, Adams R, Carnethon M, et al. Heart Disease and Stroke Statistics--2009 Update. A Report From the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 2008.
- Naghavi M, Falk E, Hecht HS, et al. From vulnerable plaque to vulnerable patient--Part III: Executive summary of the Screening for Heart Attack Prevention and Education (SHAPE) Task Force report. *Am J Cardiol* 2006; 98: 2H–15H.
- Hellings WE, Peeters W, Moll FL, et al. From vulnerable plaque to vulnerable patient: the search for biomarkers of plaque destabilization. *Trends Cardioasc Med* 2007; 17: 162–171.
- Weintraub HS. Identifying the vulnerable patient with rupture-prone plaque. *Am J Cardiol* 2008; 101: 3F–10F.
- Mallat Z, Tedgui A. Cytokines as regulators of atherosclerosis in murine models. *Curr Drug Targets* 2007; 8: 1264–1272.
- Tedgui A, Mallat Z. Cytokines in atherosclerosis: pathogenic and regulatory pathways. *Physiol Rev* 2006; 86: 515–581.
- Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med* 1999; 340: 115–126.
- Packard RR, Libby P. Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction. *Clin Chem* 2008; 54: 24–38.
- Kannel WB, Dawber TR, Kagan A, et al. Factors of risk in the development of coronary heart disease--six year follow-up experience. The Framingham Study. *Ann Intern Med* 1961; 55: 33–50.
- Assmann G, Cullen P, Schulte H. The Munster Heart Study (PROCAM). Results of follow-up at 8 years. *Eur Heart J* 1998; 19 (Suppl A): A2–11.
- Aksoh KO, Schaper A, Cogbill C, et al. Preventing myocardial infarction in the young adult in the first place: how do the National Cholesterol Education Panel III guidelines perform? *J Am Coll Cardiol* 2003; 41: 1475–1479.
- Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *J Am Med Assoc* 2001; 285: 2486–2497.
- Sheridan S, Pignone M, Mulrow C. Framingham-based tools to calculate the global risk of coronary heart disease: a systematic review of tools for clinicians. *J Gen Intern Med* 2003; 18: 1039–1052.
- Assmann G, Cullen P, Schulte H. Simple scoring scheme for calculating the risk of acute coronary events based on the 10-year follow-up of the prospective cardiovascular Munster (PROCAM) study. *Circulation* 2002; 105: 310–315.
- Buffon A, Biasucci LM, Liuzzo G, et al. Widespread coronary inflammation in unstable angina. *N Engl J Med* 2002; 347: 5–12.
- Burke AP, Farb A, Malcom GT, et al. Coronary risk factors and plaque morphology in men with coronary disease who died suddenly. *N Engl J Med* 1997; 336: 1276–1282.

22. Mann J, Davies MJ. Mechanisms of progression in native coronary artery disease: role of healed plaque disruption. *Heart* 1999; 82: 265–268.
23. Goldstein JA, Demetriou D, Grines CL, et al. Multiple complex coronary plaques in patients with acute myocardial infarction. *N Engl J Med* 2000; 343: 915–922.
24. Nissen SE. Who is at risk for atherosclerotic disease? Lessons from intravascular ultrasound. *Am J Med* 2002; 112 (Suppl 8A): 27S–33S.
25. Berk BC, Weintraub WS, Alexander RW. Elevation of C-reactive protein in „active“ coronary artery disease. *Am J Cardiol* 1990; 65: 168–172.
26. Liuzzo G, Biasucci LM, Gallimore JR, et al. The prognostic value of C-reactive protein and serum amyloid A protein in severe unstable angina. *N Engl J Med* 1994; 331: 417–424.
27. Lowe GD, Pepys MB. C-reactive protein and cardiovascular disease: weighing the evidence. *Curr Atheroscler Rep* 2006; 8: 421–428.
28. Danesh J, Erqou S, Walker M, et al. The Emerging Risk Factors Collaboration: analysis of individual data on lipid, inflammatory and other markers in over 1.1 million participants in 104 prospective studies of cardiovascular diseases. *Eur J Epidemiol* 2007; 22: 839–869.
29. Tracy RP, Lemaitre RN, Psaty BM, et al. Relationship of C-reactive protein to risk of cardiovascular disease in the elderly. Results from the Cardiovascular Health Study and the Rural Health Promotion Project. *Arterioscler Thromb Vasc Biol* 1997; 17: 1121–1127.
30. Ridker PM, Buring JE, Shih J, et al. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. *Circulation* 1998; 98: 731–733.
31. Jager A, van Hinsbergh VW, Kostense PJ, et al. von Willebrand factor, C-reactive protein, and 5-year mortality in diabetic and nondiabetic subjects: the Hoorn Study. *Arterioscler Thromb Vasc Biol* 1999; 19: 3071–3078.
32. Mendall MA, Strachan DP, Butland BK, et al. C-reactive protein: relation to total mortality, cardiovascular mortality and cardiovascular risk factors in men. *Eur Heart J* 2000; 21: 1584–1590.
33. Casas JP, Shah T, Hingorani AD, et al. C-reactive protein and coronary heart disease: a critical review. *J Intern Med* 2008; 264: 295–314.
34. Hanriot D, Bello G, Ropars A, et al. C-reactive protein induces pro- and anti-inflammatory effects, including activation of the liver X receptor alpha, on human monocytes. *Thromb Haemost* 2008; 99: 558–569.
35. Danesh J, Wheeler JG, Hirschfield GM, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med* 2004; 350: 1387–1397.
36. Ridker PM, Rifai N, Stampfer MJ, et al. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* 2000; 101: 1767–1772.
37. Ikonomidis I, Stamatelopoulos K, Lekakis J, et al. Inflammatory and non-invasive vascular markers: the multimarker approach for risk stratification in coronary artery disease. *Atherosclerosis* 2008; 199: 3–11.
38. Tan J, Hua Q, Gao J, et al. Clinical implications of elevated serum interleukin-6, soluble CD40 ligand, metalloproteinase-9, and tissue inhibitor of metalloproteinase-1 in patients with acute ST-segment elevation myocardial infarction. *Clin Cardiol* 2008; 31: 413–418.
39. Oren H, Erbay AR, Balci M, et al. Role of novel biomarkers of inflammation in patients with stable coronary heart disease. *Angiology* 2007; 58: 148–155.
40. Inoue T, Komoda H, Nonaka M, et al. Interleukin-8 as an independent predictor of long-term clinical outcome in patients with coronary artery disease. *Int J Cardiol* 2008; 124: 319–325.
41. Ikonomidis I, Andreotti F, Economou E, et al. Increased proinflammatory cytokines in patients with chronic stable angina and their reduction by aspirin. *Circulation* 1999; 100: 793–798.
42. Saremi A, Anderson RJ, Luo P, et al. Association between IL-6 and the extent of coronary atherosclerosis in the veterans affairs diabetes trial (VADT). *Atherosclerosis* 2009; 203: 610–614.
43. Gotsman I, Stabholz A, Planer D, et al. Serum cytokine tumor necrosis factor-alpha and interleukin-6 associated with the severity of coronary artery disease: indicators of an active inflammatory burden? *Isr Med Assoc J* 2008; 10: 494–498.
44. Wang TJ, Gona P, Larson MG, et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. *N Engl J Med* 2006; 355: 2631–2639.
45. Braunwald E. Biomarkers in heart failure. *N Engl J Med* 2008; 358: 2148–2159.
46. Pasterkamp G, Daemen M. Circulating cells: the biofactory for markers of atherosclerotic disease. *Eur Heart J* 2008; 29: 2701–2702.
47. Schirmer SH, Fledderus JO, van der Laan AM, et al. Suppression of inflammatory signaling in monocytes from patients with coronary artery disease. *J Mol Cell Cardiol* 2009; 46: 177–185.
48. Ganesh SK, Skelding KA, Mehta L, et al. Rationale and study design of the CardioGene Study: genomics of in-stent restenosis. *Pharmacogenomics* 2004; 5: 952–1004.
49. Patino WD, Kang JG, Matoba S, et al. Atherosclerotic plaque macrophage transcriptional regulators are expressed in blood and modulated by tristetraprolin. *Circ Res* 2006; 98: 1282–1289.
50. Hurks R, Peeters W, Derksen WJ, et al. Biobanks and the search for predictive biomarkers of local and systemic outcome in atherosclerotic disease. *Thromb Haemost* 2009; 101: 48–54.
51. Cleutjens KB, Faber BC, Rousch M, et al. Non-invasive diagnosis of ruptured peripheral atherosclerotic lesions and myocardial infarction by antibody profiling. *J Clin Invest* 2008; 118: 2979–2985.
52. Narula J, Garg P, Achenbach S, et al. Arithmetic of vulnerable plaques for noninvasive imaging. *Nat Clin Pract Cardiovasc Med* 2008; 5 (Suppl 2): S2–10.
53. Naghavi M, Falk E, Hecht HS, et al. The First SHAPE (Screening for Heart Attack Prevention and Education) Guideline. *Crit Pathw Cardiol* 2006; 5: 187–190.
54. Sitzer M. Atherosclerotic carotid stenosis and occlusion. *Front Neurol Neurosci* 2006; 21: 36–56.
55. Little WC, Constantinescu M, Applegate RJ, et al. Can coronary angiography predict the site of a subsequent myocardial infarction in patients with mild-to-moderate coronary artery disease? *Circulation* 1988; 78: 1157–1166.
56. Ambrose JA, Tannenbaum MA, Alexopoulos D, et al. Angiographic progression of coronary artery disease and the development of myocardial infarction. *J Am Coll Cardiol* 1988; 12: 56–62.
57. Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. North American Symptomatic Carotid Endarterectomy Trial Collaborators. *N Engl J Med* 1991; 325: 445–453.
58. Randomised trial of endarterectomy for recently symptomatic carotid stenosis: final results of the MRC European Carotid Surgery Trial (ECST). *Lancet* 1998; 351: 1379–1387.
59. Budoff MJ, Achenbach S, Blumenthal RS, et al. Assessment of coronary artery disease by cardiac computed tomography: a scientific statement from the American Heart Association Committee on Cardiovascular Imaging and Intervention, Council on Cardiovascular Radiology and Intervention, and Committee on Cardiac Imaging, Council on Clinical Cardiology. *Circulation* 2006; 114: 1761–1791.
60. Mitsutake R, Niimura H, Miura S, et al. Clinical significance of the coronary calcification score by multidetector row computed tomography for the evaluation of coronary stenosis in Japanese patients. *Circ J* 2006; 70: 1122–1127.
61. Schroeder S, Kuettner A, Leitritz M, et al. Reliability of differentiating human coronary plaque morphology using contrast-enhanced multislice spiral computed tomography: a comparison with histology. *J Comput Assist Tomogr* 2004; 28: 449–454.
62. Inoue F, Sato Y, Matsumoto N, et al. Evaluation of plaque texture by means of multislice computed tomography in patients with acute coronary syndrome and stable angina. *Circ J* 2004; 68: 840–844.
63. Miller JM, Rochitte CE, Dewey M, et al. Diagnostic performance of coronary angiography by 64-row CT. *N Engl J Med* 2008; 359: 2324–2336.
64. Ropers D, Rixe J, Anders K, et al. Usefulness of the multidetector row spiral computed tomography with 64-x 0.6-mm collimation and 330-ms rotation for the noninvasive detection of significant coronary artery stenoses. *Am J Cardiol* 2006; 97: 343–348.
65. Fine JJ, Hopkins CB, Ruff N, et al. Comparison of accuracy of 64-slice cardiovascular computed tomography with coronary angiography in patients with suspected coronary artery disease. *Am J Cardiol* 2006; 97: 173–174.
66. Dodd JD, Rieber J, Pomerantsev E, et al. Quantification of nonculprit coronary lesions: comparison of cardiac 64-MDCT and invasive coronary angiography. *AJR Am J Roentgenol* 2008; 191: 432–438.
67. Brenner DJ, Hall EJ. Computed tomography—an increasing source of radiation exposure. *N Engl J Med* 2007; 357: 2277–2284.
68. Yuan C, Mitsumori LM, Beach KW, et al. Carotid atherosclerotic plaque: noninvasive MR characterization and identification of vulnerable lesions. *Radiology* 2001; 221: 285–299.
69. Kwong RY, Chan AK, Brown KA, et al. Impact of unrecognized myocardial scar detected by cardiac magnetic resonance imaging on event-free survival in patients presenting with signs or symptoms of coronary artery disease. *Circulation* 2006; 113: 2733–2743.
70. Trivedi RA, Mallawarachi C, JM UK-I, et al. Identifying inflamed carotid plaques using in vivo USPIO-enhanced MR imaging to label plaque macrophages. *Arterioscler Thromb Vasc Biol* 2006; 26: 1601–1606.
71. Tang T, Howarth SP, Miller SR, et al. Assessment of inflammatory burden contralateral to the symptomatic carotid stenosis using high-resolution ultrasmall, superparamagnetic iron oxide-enhanced MRI. *Stroke* 2006; 37: 2266–2270.
72. Okane K, Ibaraki M, Toyoshima H, et al. 18F-FDG accumulation in atherosclerosis: use of CT and MR coregistration of thoracic and carotid arteries. *Eur J Nucl Med Mol Imaging* 2006; 33: 589–594.
73. Nahrendorf M, Zhang H, Hembrador S, et al. Nanoparticle PET-CT imaging of macrophages in inflammatory atherosclerosis. *Circulation* 2008; 117: 379–387.
74. Raylman RR, Wahl RL. Evaluation of ion-implanted-silicon detectors for use in intraoperative positron-sensitive probes. *Med Phys* 1996; 23: 1889–1895.
75. Nabi HA, Zubeldia JM. Clinical applications of (18)F-FDG in oncology. *J Nucl Med Technol* 2002; 30: 3–9; quiz 10–1.
76. Ogawa M, Ishino S, Mukai T, et al. (18)F-FDG accumulation in atherosclerotic plaques: immunohistochemical and PET imaging study. *J Nucl Med* 2004; 45: 1245–1250.
77. Chen W, Bural GG, Torigian DA, et al. Emerging role of FDG-PET/CT in assessing atherosclerosis in

- large arteries. *Eur J Nucl Med Mol Imaging* 2009; 36: 144–151.
78. Groen HC, Gijzen FJ, van der Lugt A, et al. High shear stress influences plaque vulnerability Part of the data presented in this paper were published in *Stroke* 2007;38:2379–81. *Neth Heart J* 2008; 16: 280–283.
79. Huang X, Yang C, Yuan C, et al. Patient-Specific Artery Shrinkage and 3D Zero-Stress State in Multi-Component 3D FSI Models for Carotid Atherosclerotic Plaques Based on In Vivo MRI Data. *Mol Cell Biomech* 2009; 6: 121–134.
80. Kerwin WS, Liu F, Yarnykh V, et al. Signal features of the atherosclerotic plaque at 3.0 Tesla versus 1.5 Tesla: impact on automatic classification. *J Magn Reson Imaging* 2008; 28: 987–995.
81. Tang D, Yang C, Mondal S, et al. A negative correlation between human carotid atherosclerotic plaque progression and plaque wall stress: in vivo MRI-based 2D/3D FSI models. *J Biomech* 2008; 41: 727–736.
82. Yuan C, Oikawa M, Miller Z, et al. MRI of carotid atherosclerosis. *J Nucl Cardiol* 2008; 15: 266–275.
83. Zhao X, Miller ZE, Yuan C. Atherosclerotic plaque imaging by carotid MRI. *Curr Cardiol Rep* 2009; 11: 70–77.
84. Davies MJ. The contribution of thrombosis to the clinical expression of coronary atherosclerosis. *Thromb Res* 1996; 82: 1–32.
85. Spuentrup E, Buecker A, Katoh M, et al. Molecular magnetic resonance imaging of coronary thrombosis and pulmonary emboli with a novel fibrin-targeted contrast agent. *Circulation* 2005; 111: 1377–1382.
86. Botnar RM, Buecker A, Wiethoff AJ, et al. In vivo magnetic resonance imaging of coronary thrombosis using a fibrin-binding molecular magnetic resonance contrast agent. *Circulation* 2004; 110: 1463–1466.
87. Overoye-Chan K, Koerner S, Looby RJ, et al. EP-2104R: a fibrin-specific gadolinium-Based MRI contrast agent for detection of thrombus. *J Am Chem Soc* 2008; 130: 6025–6039.
88. Spuentrup E, Botnar RM, Wiethoff AJ, et al. MR imaging of thrombi using EP-2104R, a fibrin-specific contrast agent: initial results in patients. *Eur Radiol* 2008; 18: 1995–2005.
89. von zur Muhlen C, von Elverfeldt D, Moeller JA, et al. Magnetic resonance imaging contrast agent targeted toward activated platelets allows in vivo detection of thrombosis and monitoring of thrombolysis. *Circulation* 2008; 118: 258–267.
90. Kolodgie FD, Gold HK, Burke AP, et al. Intraplaque hemorrhage and progression of coronary atheroma. *N Engl J Med* 2003; 349: 2316–2325.
91. Sluimer JC, Gasc JM, van Wanroij JL, et al. Hypoxia, hypoxia-inducible transcription factor, and macrophages in human atherosclerotic plaques are correlated with intraplaque angiogenesis. *J Am Coll Cardiol* 2008; 51: 1258–1265.
92. Virmani R, Kolodgie FD, Burke AP, et al. Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. *Arterioscler Thromb Vasc Biol* 2005; 25: 2054–2061.
93. O'Malley SM, Vavuranakis M, Naghavi M, et al. Intravascular ultrasound-based imaging of vasa vasorum for the detection of vulnerable atherosclerotic plaque. *Med Image Comput Comput Assist Interv Int Conf Med Image Comput Comput Assist Interv* 2005; 8: 343–351.
94. Kerwin W, Hooker A, Spilker M, et al. Quantitative magnetic resonance imaging analysis of neovascularity volume in carotid atherosclerotic plaque. *Circulation* 2003; 107: 851–856.
95. Burtea C, Laurent S, Murariu O, et al. Molecular imaging of alpha v beta3 integrin expression in atherosclerotic plaques with a mimetic of RGD peptide grafted to Gd-DTPA. *Cardiovasc Res* 2008; 78: 148–157.