From Vulnerable Plaque to Vulnerable Patient: The Search for Biomarkers of Plaque Destabilization

Willem E. Hellings, Wouter Peeters, Frans L. Moll, and Gerard Pasterkamp

There is a strong need for biomarkers to identify patients at risk for future cardiovascular events related with progressive atherosclerotic disease. Ideally, increasing knowledge of the mechanisms of atherosclerotic plaque destabilization should be translated in clinical practice. Currently, the following commonly followed strategies can be identified with the objective to detect either the local vulnerable plaque that is prone to rupture and gives rise to a thrombotic occlusion, or the systemic vulnerable patient, who has a high probability to suffer from an adverse clinical event. On the one hand, studies are ongoing to determine local atherosclerotic plaque characteristics to predict future local plaque rupture and subsequent vascular thrombosis. Newly developed imaging modalities are being developed and validated to detect these plaques in vivo. On the other hand, systemic approaches are pursued to discover serum biomarkers that are applicable to define patients at risk for future cardiovascular events. We propose a third original approach that is optional but yet unexplored, that is, to use local plaque characteristics as a biomarker not just for local plaque destabilization but for future cardiovascular events due to plaque progression in any vascular system. This review aims to provide an overview of the current standings of the identification of the vulnerable plaque and the vulnerable patient. (Trends Cardiovasc Med 2007;17:162–171) © 2007, Elsevier Inc.
plaque characteristics. This involves the noninvasive and invasive determination of atherosclerotic plaque characteristics that are considered to destabilize plaques and cause subsequent clinical events. Second, serologic biomarkers are being investigated as a measure of patient vulnerability, that is, to identify those who are more prone to suffer from a cardiovascular event.

This review will provide an overview of the current standing of the identification of the vulnerable plaque and patient, based on the current knowledge of the pathophysiology of atherosclerosis. In addition, novel strategies to identify biomarkers identifying patients at risk will be discussed.

- The Classically Defined Vulnerable Plaque

It has been established that acute clinical manifestations of atherosclerotic disease, such as myocardial infarction or stroke, are not the result of slowly progressing luminal narrowing. Instead, these are a consequence of acute disruption (rupture or erosion) of the atherosclerotic plaque, leading to exposure of thrombogenic plaque components to the bloodstream, with superimposed thrombus formation (Lee and Libby, 1997). The newly formed thrombus suddenly accelerates the degree of luminal stenosis or may totally occlude the lumen, giving rise to a myocardial infarction. On the other hand, superimposed thrombus can cause distal embolization, for example, in symptomatic atherosclerotic carotid disease (Spagnoli et al. 2004).

Histologic examination of atherosclerotic plaques obtained postmortem or during endarterectomy has identified plaque characteristics associated with adverse clinical events. When these characteristics are found in asymptomatic plaques, they are thought to confer vulnerability to becoming symptomatic of the plaque. Accordingly, the vulnerable plaque, also referred to as unstable plaque or high-risk plaque, is defined as a plaque with a high risk to cause local thrombosis and thereby unstable clinical syndromes such as unstable angina, myocardial infarction, or cerebral vascular accidents (Schaar et al. 2004). There are basically three types of vulnerable plaques: First, the typical vulnerable plaque is described as the plaque prone to rupture. It has a large lipid core that is covered by a fragile fibrous cap. Rupture of this cap exposes the contents of the lipid pool to the blood, which can trigger local thrombosis. Second, the plaque with superficial erosion is considered vulnerable. The loss of endothelial coverage in eroded plaques results in direct contact of the blood with the underlying connective tissue, which can lead to thrombus formation. Third, the plaque with a calcified nodule protruding into the lumen is considered at high risk to induce thrombus. The classically defined vulnerable plaque, or rupture prone plaque, which is the most common vulnerable phenotype, will be discussed in more detail. It is described by a well-defined set of histopathologic features, such as a large lipid core, presence of inflammatory cells, and paucity of smooth muscle cells and fibrous tissue (Table 1).

First, the rupture-prone vulnerable plaque has a large lipid core, which is composed of cholesterol and lipids remaining from death foam cells (Davies et al. 1993). It is the most thrombogenic part of the atherosclerotic plaque that contains oxidized lipids, and it is loaded with tissue factor produced by macrophages (Fernandez-Ortiz et al. 1994). The amount of fibrous tissue, such as collagen, which is also related to the cap thickness, is proportionally less. Autopsy series have shown that the size of the lipid core is bigger in ruptured plaques than in nonruptured plaques (Virmani et al. 2000). A large lipid core is disadvantageous from a biomechanical point of view because the soft core is unable to carry the mechanical forces on the plaque, which then are concentrated in the fibrous cap overlying the atheroma (Richardson et al. 1989).

Second, thinning of the fibrous cap increases vulnerability to plaque rupture. Strongly decreased cap thickness is observed near the site of rupture (Burke et al. 1997). The cap consists of extracellular matrix components such as collagen, proteoglycans, and elastin, and harbors smooth muscle cells and inflammatory cells. The thickness of the cap is the net effect of the balance between matrix synthesis by smooth muscle cells and matrix degradation by protease activity and local inflammation. Different factors contribute toward thinning of the cap in advanced atherosclerotic plaques: First, matrix synthesis is diminished because of decreased smooth muscle cell content, possibly caused by inflammation-induced apoptosis (Bennett et al. 1995). Second, matrix breakdown is increased as a result of overproduction of proteases by inflammatory cells.

Third, infiltration with inflammatory cells is another hallmark of the classically defined vulnerable plaque, especially infiltration of macrophages, which vastly outnumber other inflammatory cells like mast cells, T-cells, and neutrophils. Macrophage infiltration of the fibrous cap is associated with rupture (Friedman, 1971). They weaken the fibrous cap by secretion and activation of matrix-degrading proteases, leading to disruption of the balance between these proteases and their natural inhibitors such as tissue inhibitors of metalloproteinases and cystatin. Proteases, such as matrix metalloproteinase (MMP)-1, MMP-3, MMP-8, and MMP-9, are highly overexpressed in vulnerable and ruptured plaques compared with the stable plaques, and they are particularly active.

### Table 1. Histologic characteristics of the vulnerable plaque

<table>
<thead>
<tr>
<th>Vulnerable plaque (rupture prone)</th>
<th>Stable plaque</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large lipid core (&gt;40% of the plaque)</td>
<td>Small or absent lipid core</td>
</tr>
<tr>
<td>Thin fibrous cap, depleted of smooth muscle cells</td>
<td>Thick fibrous cap or no necrotic core</td>
</tr>
<tr>
<td>Infiltration of inflammatory cells, particularly macrophages</td>
<td>Minor infiltration of inflammatory cells</td>
</tr>
<tr>
<td>Neovascularization, intraplaque hemorrhage</td>
<td>No neovascularization, no intraplaque hemorrhage</td>
</tr>
<tr>
<td>Outward remodeling</td>
<td>No remodeling or constrictive remodeling</td>
</tr>
</tbody>
</table>
in the vulnerable regions of the plaque, such as the cap (Galis et al. 1994).

Fourth, vulnerable plaques are characterized by intimal neovascularization. Intraplaque angiogenesis contributes to plaque formation, plaque progression, and intraplaque hemorrhages, which ensue as a consequence of rupture of neovessels, in some cases, followed by plaque rupture (Barger and Beecwkes III, 1990). Neovascularization also plays a crucial role in the recruitment of inflammatory cells into the plaque (De Boer et al. 1999). In addition, intraplaque bleeding serves as a mechanism for build-up of the lipid core because the erythrocyte membranes contain large amounts of cholesterol (Kolodgie et al. 2003).

Finally, outward remodeling is considered as an additional characteristic of the vessel hiding the vulnerable plaque. Remodeling is an adaptive process that preserves the patency of the lumen when a plaque is formed. Although this seems beneficial, the presence of outward remodeling is associated with unstable phenomena such as inflammation and large necrotic core size, and leads to greater biomechanical stress on the fibrous cap (Pasterkamp et al. 1998a; Imoto et al. 2005).

### Imaging Tools to Detect the Vulnerable Plaque in vivo

Imaging techniques that enable visualization of structural and morphologic characteristics of the atherosclerotic plaque may be helpful to identify the characteristics of the vulnerable plaque in vivo and subsequently predict clinical outcome. These techniques either assess morphologic characteristics of plaques or functional properties such as strain on the fibrotic cap (elastography) or macrophage infiltration (molecular imaging). We will shortly quote the most described techniques to characterize atherosclerotic plaques (Table 2).

Duplex ultrasound is a generally available imaging modality that is able to provide insights in lesion size, degree of obstruction at the site of the lesion, and intima-media thickness. Moreover, this technique is able to detect necrotic core size on the basis of plaque echogenicity: hyperechoic homogeneous plaques are more fibrous, whereas hypoechoic plaques are associated with a large lipid core (Gronholdt et al. 1998, Van Damme and Vivario, 1993, European Carotid Plaque Study Group, 1995).

Computed tomography (CT) is another noninvasive imaging that has multiple applications: besides noninvasive detection of arterial stenosis (non-invasive CT angiography), it is well capable of detecting calcifications. Coronary CT enables computation of the coronary calcification score, which can be used as a measure of progression of atherosclerotic disease (Agatston et al. 1990). Despite the excellent detection of calcifications that correlates well with histopathologic findings (sensitivity of 79%–100% and specificity of 95% in coronary arteries) (Lau et al. 2005, Becker et al. 2003), it has limited ability to identify other morphologic plaque characteristics such as size of the lipid core and thickness of the fibrous cap (Becker et al. 2003).

Magnetic resonance imaging (MRI) is an emerging technique that can identify a range of important aspects of the atherosclerotic lesion, such as plaque size, lipid core size, calcifications, fibrous tissue, and thickness of the fibrous cap (Hatsukami et al. 2000). Puppini et al. (2006) have identified the lipid core with a sensitivity and specificity of 91.6% and 95%, respectively. This modality could also identify calcifications with a sensitivity of 80% and a specificity of 94%. Furthermore, fibrous components can be identified with a sensitivity of 83% and a specificity of 81% by this modality (Clarke et al. 2006).

Intravascular ultrasound (IVUS) is a catheter-based imaging technique that provides high-resolution ultrasound (30 MHz) images of both the lumen and the wall of an artery. It is the only imaging modality that provides images in which variations in arterial geometry and atherosclerotic plaque along the artery can be observed simultaneously in vivo (Nighoghssian et al. 2005). Besides revealing information of the lumen area, plaque area, and vessel area, this imaging modality may identify morphologic plaque components (lipid core, calcifications, and fibrosis) through differences in echogenicity (Nissen and Yock, 2001).

Optical coherence tomography (OCT) is a promising new technique capable of accurate detection of plaque composition (sensitivity and specificity for necrotic core size >90%) (Yabushita et al. 2002). Fibrous tissue can be detected with a sensitivity of 79% and specificity of 99% (Kume et al. 2006). Because of its high resolution, it is also capable of identifying macrophage infiltration, showing excellent correlations with macrophage infiltration determined by histology (Tearney et al. 2003). However, penetration depth is limited.

Intravascular ultrasound–based elastography has been introduced to assess the local mechanical (elastic) properties of the arterial wall. The rationale of this technique is that tissue elements differing in hardness will be compressed differently by mechanical pressures. Hard tissues, containing collagen or calcifications, are compressed less compared with soft lipid–rich tissue. Schaar et al. (2003) have demonstrated in a postmortem study that intravascular elastography can accurately diagnose vulnerable plaques (large lipid core, thin cap, and heavy macrophage infiltration), with positive predictive value of 88% and negative predictive value of 89%.

### Molecular Imaging

The conventional imaging techniques are particularly based on anatomic and physiologic heterogeneity to provide image differences. Molecular imaging will help to identify specific molecular targets, pathways, and molecular processes by using radioactive-labeled molecules, which are specifically associated with the destabilization of the atherosclerotic plaque (Jaffer and Weissleder, 2005). The identification of specific molecular atherosclerotic radiolabeled markers with different imaging techniques (MRI, positron emission tomography, or single photon emission CT) enables detection of the disease in an early stage and discrimination between active and inactive elements and stages of the disease. Examples of targets for molecular imaging are macrophages and markers for apoptosis, both of which are thought to be important in plaque destabilization. Animal studies have demonstrated that contrast-enhanced MRI with iron oxide particles shows characteristic signal intensity changes that correlate with iron accumulation within intraplaque macrophages. In a prospective study, Trivedi et al. (2004) have identified intraplaque macrophages by contrast-enhanced MRI in symptomatic patients. Imaging of apoptosis in carotid atherosclerotic plaques
Table 2. Imaging techniques to detect the vulnerable plaque

<table>
<thead>
<tr>
<th>Precision of detection</th>
<th>Duplex</th>
<th>CT</th>
<th>MRI</th>
<th>IVUS</th>
<th>OCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid core +</td>
<td>Gronholdt et al., 1998</td>
<td>Becker, 2003</td>
<td>Clarke et al., 2006</td>
<td>Kume et al., 2006</td>
<td>Yabushita et al., 2002</td>
</tr>
<tr>
<td></td>
<td>ECPSG, 1995</td>
<td>73</td>
<td>67</td>
<td>59</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>78</td>
<td>78</td>
<td>97</td>
<td>90</td>
</tr>
<tr>
<td>Fibrous components 56 88</td>
<td>Van Damme and Vivario, 1993</td>
<td>Becker, 2003</td>
<td>Clarke et al., 2006</td>
<td>Kume et al., 2006</td>
<td>Yabushita et al., 2002</td>
</tr>
<tr>
<td></td>
<td>70 a</td>
<td>83</td>
<td>81</td>
<td>88</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>86 b</td>
<td>88</td>
<td>86</td>
<td>98</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>86</td>
<td>99</td>
<td>95</td>
<td>99</td>
</tr>
<tr>
<td>Calcification +</td>
<td>ECPSG, 1995</td>
<td>Becker, 2003</td>
<td>Clarke et al., 2006</td>
<td>Kume et al., 2006</td>
<td>Yabushita et al., 2002</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>86</td>
<td>99</td>
<td>98</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>79</td>
<td>95</td>
<td>95</td>
<td>97</td>
<td>79</td>
</tr>
<tr>
<td>Hemorrhage 75 87</td>
<td>Van Damme and Vivario, 1993</td>
<td>Lau et al., 2005</td>
<td>Puppini et al., 2006</td>
<td>96</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>nr</td>
<td>80</td>
<td>93</td>
<td>96</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>nr</td>
<td>92</td>
<td>100</td>
<td>nr</td>
<td>nr</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrous cap nr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spatial resolution</td>
<td>0.5–1 mm Hennerici and Neuerburg-Heusler 1998</td>
<td>0.4 mm Lee and Sagel, 1998</td>
<td>&lt;0.1 mm Edelman and Hesselink 1990</td>
<td>0.1 mm Kawase et al., 2007</td>
<td>0.01 mm Bouma et al. 2003</td>
</tr>
</tbody>
</table>

This table summarizes the sensitivity (se) and specificity (sp) of duplex ultrasound (Duplex), CT, MRI, OCT, and IVUS to identify plaque characteristics in vivo. When an imaging technique was able to detect a certain plaque characteristic, but this was not expressed in terms of sensitivity and specificity, this is indicated by a “+.” Not reported (nr) means that there are no data available. The maximum spatial resolution is the capability to clearly define different features and details within an image: a: fibrous atheroma; b: fibrous-calcified.
has been successfully performed by Kiet-selaer et al. (2004) who performed single photon emission CT–based plaque imaging using technetium-99m–labeled annexin V as a visualization agent to detect apoptosis in atherosclerotic plaques in vivo. Patients with recently symptomatic plaques (<1 week before imaging) showed contrast enhancement by technetium-labeled annexin V, whereas plaques of patients who had not recently been symptomatic showed no contrast enhancement.

**• The Classically Defined Vulnerable Plaque: the Gold Standard to Identify Patients at Risk for Cardiovascular Events?**

Theoretically, plaque characteristics assessed by local plaque imaging may identify lesions that are considered highly vulnerable, regardless of whether the plaque causes hemodynamically significant stenosis. However, it is unknown if the previously mentioned vulnerable plaque characteristics hide positive predictive value to identify plaques that are prone to rupture. The fact that strong associations between certain vulnerable plaque characteristics and plaque rupture are observed in cross-sectional studies does suggest, but not prove, that plaques hiding these features actually have a higher risk to rupture. Prospective evidence regarding the predictive value of vulnerable plaque characteristics for future events is currently lacking. In addition, evidence from observational studies suggests that the positive predictive value of the earlier defined plaque characteristics to define vulnerable plaques at risk to cause future events may be low because (1) lipid-rich inflammatory plaques are also frequently observed in asymptomatic patients, (2) plaques lacking typical vulnerable histopathologic characteristics are able to cause clinical events, and (3) plaque rupture itself is often asymptomatic. In the next paragraphs, we will discuss these arguments shortly.

Autopsy series have revealed that lipid-rich inflammatory plaques are not only found in patients with clinical manifestations, but also in asymptomatic patients. In an autopsy series of 124 coronary arteries from elderly patients with noncardiovascular cause of death, 41% of cross-sections showed cap infiltration with macrophages in nonruptured coronary arteries. In 71% of the coronary plaques, one or more cross-sections showed cap infiltration with macrophages (Pasterkamp et al. 1999). Another post-mortem study has demonstrated that a large lipid pool and a combination of a large lipid pool and macrophage infiltration are also frequently observed in asymptomatic plaques: 25% and 20%, respectively (Vink et al. 2002). Apparently, inflammation and a large lipid core, the main hallmarks of the vulnerable plaque, are not very specific phenomena. These data suggest that the positive predictive value of these classically defined vulnerable plaque characteristics for plaque rupture and subsequent thrombotic events is likely to be low.

Another finding is that the classically defined rupture prone vulnerable plaques are not mandatory for formation of a luminal thrombus and subsequent events. In a subset of patients, especially among the young and women, clinically manifest events caused by luminal thrombosis are not triggered by plaque rupture. Instead, formation of the thrombus is initiated by a focal plaque erosion or endothelial denudation. These eroded plaques are not as heavily inflamed as the ruptured plaques and possess a more fibrous phenotype (Farb et al. 1996). Eroded plaques can also be considered vulnerable, but they lack specific features (e.g., large lipid core) that would facilitate noninvasive discrimination from none-roded plaques.

Another limitation of the use of the traditional pathologic definitions of the vulnerable plaque for patients at risk is that plaque rupture can be asymptomatic. Asymptomatic plaque ruptures in the coronary arteries have been observed in 9% of healthy patients and 22% of patients with diabetes and hypertension (Davies et al. 1989). Healed ruptures distant from the culprit lesion are a common finding in patients suffering acute coronary death (Burke et al. 2001), and almost 20% of asymptomatic carotid arteries show signs of plaque rupture at postmortem examination (Svindland and Torvik, 1988).

**• The Natural History of Plaque Rupture and the Pathologic Definition of the Vulnerable Plaque**

Although imaging techniques may be perfectly capable of revealing certain plaque characteristics, these will be of little value unless this specific imaged characteristic is prospectively shown to indicate a risk for future plaque disruption with subsequent clinical events. At present, the predictive value of the traditionally defined vulnerable plaque characteristics (large lipid core, thin fibrous cap, and macrophage infiltration) for the occurrence of adverse events is actually unknown. Studies investigating the predictive value of imaged plaque parameters have been conducted, for example, Integrated Biomarker and Imaging Study I. This study investigated IVUS, IVUS-based elastography, and angiography at baseline and after 6 months of follow-up in patients referred for percutaneous coronary intervention for stable or unstable angina (Van Mieghem et al. 2006). The main finding was a decrease in the number of high-strain spots by elastography after follow-up, but the other imaging assessments showed no changes. The number of patients (90) was too small to be able to link imaging characteristics to adverse outcomes. The PROSPECT trial is a larger trial that investigates the predictive value of IVUS, angiography, and virtual histology of the complete coronary tree for the occurrence of future cardiovascular events, with a follow-up of 2.5 years. The initial enrollment of 700 patients has been completed in 2006, and follow-up is still ongoing. Prospective plaque imaging studies will help to understand the natural history of atherosclerotic disease and may enable us to define plaque characteristics, which predict that a plaque will become symptomatic in the future. However, the use of measures like large lipid core and the presence of inflammatory cells as surrogate markers for plaque vulnerability merits careful consideration, considering the high prevalence of vulnerable plaque characteristics in non–event-related coronary arteries.

**• The Vulnerable Patient**

A problem of many imaging modalities is the capability to visualize only a certain part of the vascular tree. Because plaques, especially vulnerable plaques, tend to be associated with compensatory remodeling, many potentially dangerous plaques will not be detected by standard imaging
Thrombogenicity of the blood, and vulnerable plaques, such as vulnerable plaques, patients could be recognized by different systemic outcome (local determinants for systemic outcome, tissue epidemiology). Local plaque characteristics (morphology, protein expression, and genes) that are predictive of local outcome, natural history of plaque rupture. A novel approach will be the identification of local plaque characteristics with plaque imaging coupled to follow-up (local determinants for systemic outcome, epidemiology). An upcoming approach is the identification of vulnerable plaques with plaque imaging coupled to follow-up (local determinants for systemic outcome, epidemiology). Traditional risk factors are used to define the risk of cardiovascular events (systemic determinants to predict systemic outcome, epidemiology). An upcoming approach is the identification of vulnerable plaques with plaque imaging coupled to follow-up (local determinants for systemic outcome, tissue epidemiology). A novel approach will be the identification of local plaque characteristics (morphology, protein expression, and genes) that are predictive of systemic outcome (local determinants for systemic outcome, tissue epidemiology).

Figure 1. Overview of study designs to identify markers to predict future cardiovascular events. Traditionally, systemic risk factors are used to define the risk of cardiovascular events (systemic determinants to predict systemic outcome, epidemiology). An upcoming approach is the identification of vulnerable plaques with plaque imaging coupled to follow-up (local determinants for local outcome, natural history of plaque rupture). A novel approach will be the identification of local plaque characteristics (morphology, protein expression, and genes) that are predictive of systemic outcome (local determinants for systemic outcome, tissue epidemiology).

Techniques such as angiography (Pasterkamp et al. 1998b). Therefore, with presently available imaging techniques, one can only speculate where to look for the classically defined vulnerable plaques. In addition, as mentioned before, it is unknown if the detection of a large lipid core with inflammatory cells with an imaging modality will hold a strong promise for a future in prognostic research. Therefore, the potential of this “local determinants to predict local outcome” approach (Figure 1) may be limited.

To reliably predict if a patient will develop a cardiovascular event, we can consider many other factors besides local plaque characteristics. Systemic risk factors for atherosclerosis such as smoking, diabetes, hypercholesterolemia, sex, and age have been appreciated for a long time. In their key article, Naghavi et al. (2003) introduce the term vulnerable patients: “patients in whom disruption of a vulnerable plaque is likely to result in a clinical event.” These vulnerable patients could be recognized by different factors, such as vulnerable plaques, thrombogenicity of the blood, and vulnerability of the myocardium.

Detection of the vulnerable patient should ideally be easy to perform, with high availability and by noninvasive means. In clinical practice, the search for the vulnerable patient should be performed by a cost-effective stepwise approach. The most logical way to start is careful examination of clinical characteristics such as traditional risk factors and cholesterol levels. In this aspect, a useful diagnostic test such as the ankle brachial index should not be ignored because it is a very easy test to perform and it possesses good prognostic value (Ogren et al. 1993). Patients at high risk could then be referred for screening by noninvasive imaging techniques. This is basically the strategy as proposed by the Screening for Heart Attack Prevention and Education task force report (Naghavi et al. 2006). Essentially, the strategy as currently proposed builds upon traditional risk factors in combination with plaque measurements such as intima-media thickness and coronary calcium score.

At present, the “systemic determinants to predict systemic outcome” approach (Figure 1) enables risk stratification but is not able to discriminate between individual patients who will or will not suffer a clinical event such as a myocardial infarction or a stroke. Although screening for atherosclerosis seems promising, it may be more effective when it does not only incorporate known risk factors, but it is also extended by new specific markers identifying patients at risk for cardiovascular events. Therefore, new developments are needed to find these markers. The following section addresses developments and strategies that are used in the search of new markers.

Systemic Biomarkers to Identify Patients at Risk for Cardiovascular Events

One of the easiest new approaches that could be incorporated in clinical practice is to test a peripheral blood sample for the presence of a specific atherosclerosis marker. Different study setups have been applied to investigate such markers, (1) comparing nonatherosclerotic with atherosclerotic patients, (2) comparing stable atherosclerosis vs. unstable atherosclerosis, and (3) relating baseline values of biomarkers to future clinical events. The latter is the most laborious study design, but also by far the most appropriate to test the value of a biomarker to identify vulnerable patients. Because atherosclerosis is a systemic inflammatory disease, known inflammatory proteins and acute phase proteins have been examined to identify atherosclerotic lesions. The most extensively studied serum biomarker in atherosclerotic disease is C-reactive protein (CRP), often referred to as high-sensitivity CRP. Presence of this acute-phase protein in the serum is greatly increased after infection or trauma, but in the absence of these events, it has a value as a surrogate biomarker for atherosclerosis. It is associated with traditional cardiovascular risk factors, such as smoking and obesity, but has an independent predictive value for the occurrence of cardiovascular events (Haverkate et al. 1997). Treatment with statins, known to reduce plaque inflammation, is followed by a decrease in high-sensitivity CRP levels (Nissen et al. 2005). Nevertheless, predictive value of CRP as a surrogate marker for atherosclerosis is only moderate (less than that of the traditional risk factors), and CRP testing has not been
widely accepted in the clinical setting (Danesh et al. 2004). In addition, many other inflammatory markers have been examined in relation to atherosclerotic disease, including interleukin 6 (IL-6), IL-18, monocyte chemoattractant protein-1, tumor necrosis factor α, soluble CD40 ligand, and immunoglobulins (Blankenberg et al. 2003a, De Lemos et al. 2003, Heeschen et al. 2003, Kovanen et al. 1998, Pai et al. 2004). However, none of these biomarkers have surpassed the predictive value of CRP, and routine clinical use cannot be recommended. A more extensive review of currently known biomarkers that are associated with atherosclerotic disease and adverse outcome can be found elsewhere (Koenig and Khuseynova, 2006).

In the search for new biomarkers, one of the approaches is to develop a serum test for molecules that are known to be present in unstable plaques or that are involved in the mechanisms of plaque destabilization. High serum levels of plaque-derived markers could represent a high burden of unstable plaques and thereby help identify the vulnerable patient. It has been shown that proteins derived from unstable plaques can be secreted into the bloodstream, especially in the case of a disrupted plaque, and thus be retrieved in the circulating blood. It was shown that in patients undergoing percutaneous coronary interventions, blood samples distal from the coronary plaque contained increased amounts of IL-6, suggesting secretion of IL-6 from the plaques (Maier et al. 2005). Interestingly, IL-6 has been identified as a possible biomarker, and IL-6 release from plaques is able to induce CRP in the liver. The family of MMPs, of which especially MMP-9 plays an important role in plaque destabilization, are an interesting target to identify the vulnerable patient. In a follow-up study, blood MMP-9 levels at baseline were found to be associated with future cardiovascular events. However, the predictive value was moderate (comparable or less than CRP) and correcting for known risk factors, and CRP disturbed the association between MMP-9 and clinical outcome (Blankenberg et al. 2003b). Another example is pregnancy-associated plasma protein A, which seems to be specifically expressed in ruptured and unstable plaques. Plasma levels of this protein strongly correlated with plaque levels, and a recent study documented an increased incidence of the combined end point of death or acute coronary syndrome in patients with chronic stable coronary artery disease (Elesber et al. 2006).

Besides being a surrogate marker for inflammation and plaque instability, biomarkers can also relate to the vulnerable blood, another ingredient of the vulnerable patient. The most widely investigated markers are D-dimer and fibrinogen (Danesh et al. 2005, Danesh et al. 2001).

The currently known biomarkers potentially offer the possibility of risk stratification, but the “magic bullet” to identify the vulnerable patient that hides many vulnerable plaques has not been found. Therefore, the search for new biomarkers is ongoing. Because atherosclerosis is a multifactorial disease, it is probably simplistic to assume that a single systemic biomarker would suffice. Instead, several biomarkers could be combined in a multimarker test. With currently available biomarkers, even the use of multiple biomarkers only adds moderate predictive value to traditional cardiovascular risk factors. A cohort of 3209 patients derived from the Framingham Heart Study was tested for 10 biomarkers, but the relative hazard for cardiovascular events during 7.4 years of follow-up was no more than 1.84 for the patients with the highest quintile of multimarker scores compared with the lowest two quintiles (Wang et al. 2006). This underlines the need for more specific prognostic biomarkers to identify the patients at high risk to suffer a cardiovascular event.

• Local Plaque Characteristics in Relation to Systemic Clinical Outcome: From Plaque to Patient

As mentioned before, previously determined characteristics of the local atherosclerotic plaque could hold a certain predictive value for local plaque rupture; however, subjects who never suffered an event also hide plaques with inflammatory properties. Alternatively, instead of searching for local plaque characteristics predicting local rupture, or a systemic biomarker predicting systemic events, there is a third option: the search for local plaque characteristics predictive of systemic cardiovascular events (“local determinants for systemic outcome”) (Figure 1). There is accumulating evidence that the processes causing plaque destabilization are not limited to a single culprit plaque but are diffusely present throughout the vascular tree. Mauriello et al. (2005) showed that in 16 patients who died from myocardial infarction, inflammation was not only evident in the culprit lesion but throughout the entire coronary tree compared with patients dying from noncardiac causes (n = 14). Plaques throughout the coronary tree, either ruptured or vulnerable (defined as fibrous cap thinner than 65 μm) but also stable (cap >65 μm), showed a threefold increase in inflammatory cell density compared with (mostly stable) plaques in the coronary tree of patients with stable angina of asymptomatic patients. Other studies have shown that changes in the vascular wall are not confined to the coronary tree. When coronary disease presents with unstable clinical symptoms, carotid plaque morphology as measured by duplex ultrasound is also more unstable. In patients with unstable angina, 23.2% of patients demonstrated an unstable carotid plaque compared with 3.2 patients with stable angina (Lombardo et al. 2004). Furthermore, a prospective study of 5393 carotid angiograms showed that patients with irregular carotid plaques had increased risk of nonstroke cardiovascular death during follow-up compared with patients with smooth plaques, which could not be explained by differences in traditional cardiovascular risk factors (Rothwell et al. 2000). These studies all lend support to the idea that instability of the vascular wall is a systemic process rather than only local inflammation, and that the molecular structure of the atherosclerotic vascular wall at one site could hold information about the stability of the whole system. Thus, theoretically, inflammatory mediators or other markers of instability measured in one territory of the vascular tree could provide a fingerprint of the degree of stability of the whole atherosclerotic arterial system. Local atherosclerotic vascular tissue, obtained by endarterectomy or atherectomy, could thus help to find markers associated with generalized plaque vulnerability of the vascular tree. This novel approach is currently being...
investigated by the Athero-Express study, which already has included atherosclerotic specimens of more than 1000 patients undergoing carotid endarterectomy to test the predictive value of plaque characteristics of locally obtained plaque specimen for the occurrence of systemic adverse cardiovascular events (Verhoeven et al. 2004). All patients undergo a 3-year follow-up. Patients fill in questionnaires and donate blood at inclusion. The study was initiated in 2002 and does not suffer from a lack of power: approximately 20% of all patients reach a hard clinical end point within 3 years.

- Technical Development and Novel Approaches to Discover New Targets to Identify the Vulnerable Patient

The use of biomarkers and new imaging tools to identify the vulnerable patient with prospectively defined plaque characteristics actually depend on current knowledge of mechanisms of atherosclerosis. The search for biomarkers as performed currently could be considered a "fishing expedition," in which candidate proteins are picked and tested for their ability to identify the vulnerable patient. This approach then brings target genes and proteins into atherosclerotic animal models and, one hopes, to clinical practice. This time-consuming approach is cumbersome and mostly involves the value of known candidate targets, and validation is hampered by the differences between atherosclerotic animal models and humans.

Advancing technology enables the study of larger numbers of genes and proteins simultaneously. The unraveling of the total human genome has stimulated the wide use of strategies that investigate cells and tissues for the total genome (genomics) or proteome (proteomics). Microarrays can be used to investigate expression of thousands of genes at once. Proteomics cover the entire protein spectrum found in humans (Elrick et al. 2006). Such an approach could very well be used to identify serum markers for atherosclerosis (Vivanco et al. 2005). However, it is still a challenge to perform proteomics studies on sera because of the large amounts of albumin and other serum proteins in comparison with relatively low expressed biomarker targets. Therefore, research on proteomic techniques should be encouraged. When these techniques have advanced sufficiently to enable reliable proteomics research on blood samples, researchers possessing serum samples of longitudinal patient cohorts should be encouraged to perform proteomics on these samples to identify new prognostic serum markers. Alternatively, proteomic studies on plaque levels may provide new biomarker targets. In the Athero-Express study, protein expression in the atherosclerotic plaque from patients undergoing carotid endarterectomy is linked to clinical follow-up after the operation. Differentially expressed proteins between cases (who develop adverse cardiovascular events during follow-up after carotid endarterectomy) and controls will be identified by proteomics on protein isolated from the carotid plaques. Selection of cases and controls is done irrespective of plaque characteristics at baseline. This approach will help us to redefine or fine-tune the definition of the plaques that make the patient vulnerable. This research will yield new target proteins and may identify protein targets that have a predictive value as a serologic marker, but more importantly, the identified target proteins can also be used for molecular imaging.

- Summary

The search for markers of the vulnerable plaque and vulnerable patient represents an exciting new research field. On the one hand, studies are ongoing to prospectively determine local atherosclerotic plaque characteristics to predict future plaque rupture and subsequent adverse events. Newly developed imaging modalities can be used to detect these plaques in vivo. On the other hand, systemic approaches are pursued to discover serum biomarkers for cardiovascular disease, which can be used to define patients at risk for future cardiovascular events. New approaches with genomics and proteomics studies provide opportunities to discover new targets to identify patients at high risk for cardiovascular events.

References


Therapeutic Approaches of Angiogenesis Inhibition: Are We Tackling the Problem at the Right Level?

Arjan W. Griffioen*

A growing body of evidence now demonstrates that inhibition of angiogenesis is a promising way for treatment of disease. Although the field of angiogenesis research is strongly linked to cancer biology, many other diseases were found to be dependent on angiogenesis as well, introducing a potential benefit from antiangiogenesis treatment. Recently, the first specific angiogenesis inhibitor was approved by the Food and Drug Administration for the treatment of colorectal cancer. Currently, several compounds with angiostatic activity are approved, and many are in late-stage clinical development. Most of these are indirect inhibitors, either clearing angiogenic growth factors from the circulation or blocking the signaling pathways activated by these growth factors. Although these compounds seem to represent an efficient strategy in cancer treatment, they possess an intrinsic trait to induce resistance. Therefore, it remains to be seen whether this strategy will be the most attractive in the future. Advancing insights into fundamental mechanisms will be necessary in the development of novel anticancer strategies based on inhibition of angiogenesis. (Trends Cardiovasc Med 2007;17:171–176) © 2007, Elsevier Inc.

• Introduction

The hypothesis that the growth of tumors is dependent on the formation of new blood vessels, put forward by Folkman in the early 1970s (Folkman 1971), indicated that angiogenesis inhibitors might be discovered and used as therapy against cancer. Not until the early 1990s were the first specific angiogenesis inhibitors described. Over the last 15 years, considerable progress has been made in the development of therapies based on targeting tumor angiogenesis (Folkman 2006; Griffioen and Molema 2000). Currently, several angiogenesis inhibitors are approved for the treatment of cancer, and many are in late-stage clinical testing.

Angiogenesis occurs through an intricately regulated cascade of processes in growing tissues where for example conditions of hypoxia have turned on the production of angiogenic growth factors such as the families of vascular endothelial cell growth factors (VEGFs) and fibroblast growth factors. Such growth factors are sensed by endothelial cells in preexisting capillaries that subsequently produce proteases to dissolve the basement membrane and extracellular matrix, thereby allowing migration of endothelial cells into the direction of the stimulus. Endothelial cells will proliferate and form new sprouts that become functional blood vessels after the attraction of accessory