Monocyte activation in atherosclerosis: the value of neopterin levels as a biomarker in cardiovascular disease (review)

Atherosclerosis is a chronic inflammatory disease. Monocytes play a key role throughout the pathogenesis of atherosclerosis. The early stages of the disease process are characterised by adhesion and transmigration of monocytes/macrophages through the endothelial layer. A chronic inflammatory response that is maintained by monocytes/macrophages in concordance with T-lymphocytes, endothelial cells and smooth muscle cells, finally results in clinical manifestations of cardiovascular disease. This review aims to provide a short introduction on the role of monocytes/macrophages in the pathogenesis of atherosclerosis followed by a review on the clinical significance of monocyte activation in patients with cardiovascular disease. We will focus on the possible role of neopterin as a marker of monocyte/macrophage activity, in predicting adverse events in patients with acute coronary syndromes.

INTRODUCTION

The complications of atherosclerotic vascular disease, such as coronary artery disease, cerebrovascular disease and peripheral artery disease are still the leading cause of morbidity and mortality in the Western society [1]. Moreover, they are emerging as a major cause of death in other parts of the world [2]. Thus, major efforts are warranted to stop this global epidemic.

Atherosclerosis is a chronic disease that is already present at an early age [3]. It slowly progresses over time and in most cases it manifests itself as coronary artery disease, stroke or peripheral artery disease in the sixth or seventh decade. However, the prevalence of coronary atherosclerosis at younger ages is much higher than one would expect: 37% of ‘healthy’ heart donors 20-29 years of age have advanced atherosclerotic lesions as detected with intravascular ultrasound, 60% of those ageing 30 to 39 years, 71% between 40-49 years and 85% of those older than 50 years old [4]. These findings emphasise the importance of early intervention and identification of patients at risk. Consequently, a thorough understanding of the pathophysiology of the disease process is warranted to develop new strategies in reducing the burden of cardiovascular disease. In the following sections we will focus on the role of monocytes/macrophages in atherosclerosis and translate their role to current and future clinical applications.

MONOCYTES IN ATHEROSCLEROSIS

Innate immunity

Inflammation is a process in which blood leukocytes leave the vascular space and enter a tissue site in response to a perceived pathogen. In humans there are two distinctive defense mechanisms that form the base of any inflammatory response: innate and adaptive immunity. Both systems have their own defense mechanisms but act in close co-operation. The innate immune system provides the fastest but least specific response to toxic or inflammatory stimuli. It forms the first line of defense and it is based on the recognition of pathogen-associated molecular patterns (PAMP). Its cellular components comprise monocytes that can transform to macrophages, dendritic cells, ‘natural killer’ cells and mast cells. The cells of the innate immune system express a limited and highly conserved repertoire of pattern-recognition receptors such as scavenger receptors and Toll-like receptors. These receptors recognize PAMPs. Once activated through contact with a PAMP or upon IFNγ stimulation by T helper type 1 lymphocytes, the cells of the innate immunity start with producing substances that mount a local inflammatory response and/or start with endocytosis and lysosomal degradation of a presented particle (e.g. cytokines, growth factors, chemokines and lysosomal enzymes such as myeloperoxidase). Moreover, through
the expression of major histocompatibility complex (MHC) class II receptors on their surface, they act as antigen-presenting cells to T lymphocytes.

Monocytes in the pathogenesis of atherosclerosis

Monocytes/macrophages, as part of the innate immune response, are key players in the pathogenesis of atherosclerosis. From the early beginning of lesion formation to the late complicated stages of the unstable or eroded plaques, the whole process seems to be dominated by a constant input of monocytes/macrophages [5-7].

The pathogenesis of atherosclerosis is currently viewed in the light of the response-to-injury hypothesis as proposed in the 1970s by Ross [8]. The central thesis is that atherosclerosis results from the response of an injured arterial wall to several noxious stimuli. While it was first suggested that the injury had a mechanical character, he later proposed that it was an inflammatory response evoked by a huge number of stressors (atherogenic risk factors) that lead to the atherosclerotic changes in the vessel wall [5]. It is nowadays widely accepted that a chronic inflammatory response is the result of an interaction between genetic and environmental risk factors (especially modified lipoproteins), monocytes/macrophages, T lymphocytes and the normal cellular elements of the vessel wall such as endothelial cells and smooth muscle cells [9].

The first stage of atherosclerosis is called the fatty streak. Already at this stage monocytes are indispensable for lesion formation. The first observations that monocytes played an active role in atherogenesis were reported by Gerrity, who not only discovered that fatty streaks were the result of monocyte accumulation from the blood stream to the subendothelium [10], but also that these monocytes progressed to the lipid laden foam cells [11]. Due to stimuli such as atherogenic risk factors (‘injury’), the endothelium becomes activated and under-
impossible to measure the activity of monocytes/macrophages at the site of the atherosclerotic plaque in living patients. Therefore, most of the clinical research on monocyte/macrophage activity has been done in an indirect manner, either by histopathological studies of coronary arteries, by examining monocytes from the peripheral circulation or by serological studies measuring products of activated monocytes/macrophages such as chemokines, cytokines or neopterin. Much of our knowledge of the pathophysiology the acute coronary syndromes is derived from histopathological studies. Especially the introduction of the atherectomy catheter has provided an excellent opportunity to study samples of atherosclerotic plaque. By studying atherectomy samples it was learned that especially plaques from patients with unstable angina pectoris and non Q-wave myocardial infarction are characterised by large amounts of monocytes/macrophages [15] when compared to patients with stable atherosclerosis. Moreover, it has become clear that the extent of plaque inflammation predicts recurrent unstable angina in patients who underwent a direct coronary atherectomy [16]. Thus, essential information on pathophysiological mechanisms underlying complicated atherosclerotic diseases may be derived from studies using this technique.

**NEOPTERIN**

First isolated from larvae of bee in 1963 [17], neopterin has since then emerged as a marker of an enhanced cell-mediated immune response in a broad spectrum of clinical disease entities. Eventually, neopterin was identified as the fluorescent component that was elevated in the urine of mice with Ehrlich ascites tumor [18]. Somewhat later, it was demonstrated that neopterin was elevated in the urine of patients with various malignant disorders or viral infections [19]. Nowadays it is clear that neopterin can be detected in most body fluids [20].

Neopterin is the exclusive product of monocytes/macrophages that have been stimulated by interferon-γ, a cytokine that is produced by activated T-lymphocytes and natural killer-cells [21]. Therefore, neopterin provides an ideal diagnostic tool for monitoring the cell-mediated innate immune response in clinical disease.

**Biochemistry of neopterin**

Neopterin is synthesised *in vivo* by activated monocytes from guanosine triphosphate (GTP) via GTP cyclohydrolase I (GTP-CH). The activity of GTP-CH can be greatly enhanced by interferon-γ [22;23]. 7,8-dihydroneopterin triphosphate (NH₂TP) is on the biosynthetic pathway of 5,6,7,8-tetrahydrobiopterin (BH₄). BH₄ represents the electron donor in the hydroxylation of phenylalanine to tyrosine in the liver and of tyrosine to L-dopa and tryptophan into 5-hydroxy-tryptophan in neuroendocrine tissue synthesizing catecholamines or serotonin. Human monocytes/macrophages lack the enzyme 6-pyruvoyl-tetrahydrobiopterin synthetase which converts NH₂TP to 6-pyruvoyl-tetrahydrobiopterin synthetase which converts NH₂TP to 6-pyruvoyl-tetrahydrobiopterin thus, in these cells NH₂TP accumulates and, after hydrolysis by phosphatases, is excreted as dihydroneopterin or neopterin (figure 1) [23-25]. On the basis of biochemical *in vitro* evidence it has been concluded that increased neopterin biosynthesis during inflammation is primarily derived from interferon-γ activated monocytes/macrophages. Although IFN-γ is the most potent inducer of neopterin production, neopterin is also synthesised upon stimulation of monocytes/macrophages by TNF-α and lipopolysaccharide [26].

**The physiological role of neopterin**

The exact physiological role of neopterin is still unclear. It is suggested that neopterin acts as an endogenous inhibitor of folate synthesis by intracellular pathogenic micro-organisms [27]. *In vitro* experiments
Neopterin in cardiovascular disease

Figure 1. Neopterin production by the monocyte/macrophage.

have suggested a role for neopterin in oxygen free-radical mediated processes, apoptosis and the activation of redox-sensitive transcription factors [28-30]. Importantly, it was demonstrated that neopterin attenuated the oxidation of low density lipoprotein by Cu\(^{++}\) and peroxynitrite [31]. Also, it stimulates the gene expression of inducible nitric oxide synthetase (iNOS) in vascular smooth muscle cells [32]. Thus, it is likely that the main physiological function of neopterin is that of an anti-oxidant. However, unlike its reduced form, 7,8-dihydroneopterin, neopterin does not function as a scavenger of free radicals.

Factors influencing serum neopterin levels

Serum levels of neopterin can be easily assessed but when applying the test some considerations with respect to factors that influence serum neopterin levels should be made.

Schennach et al. reported the relation between neopterin levels, and clinical and environmental parameters in 1156 healthy blood donors [33]. They and others [34-36] found a rather strong correlation of neopterin levels with age. Also a correlation was found with diastolic blood pressure and a much weaker relation with body mass index. Importantly, no relation was found with serum cholesterol levels.

Neopterin testing in cardiovascular disease

As mentioned before, the main cause of cardiovascular disease, atherosclerosis, is characterised as a low-grade inflammatory process. Since a major pathophysiological role is attributed to the infiltration, activation and proliferation of monocytes/macrophages, several authors have investigated the role of neopterin in atherosclerotic cardiovascular disease.

Cardiovascular risk factors

Although the major cardiovascular risk factors are known to induce inflammation and oxidative stress at the level of the vessel wall, there are only few data relating neopterin with cardiovascular risk factors. Low density lipoproteins, especially the oxidised form oxLDL, are known to be potent inducers of an inflammatory response [14]. In vitro, it was demonstrated that the HMG-CoA inhibitor atorvastatin, reduced neopterin formation in both peripheral blood monocytes and cultured monocytes/macrophages [37]. Results from two clinical trials were not conclusive. We demonstrated a decrease of neopterin levels, in patients with established atherosclerosis, after three months of treatment with fluvastatin, but this effect had disappeared after one year [38]. Gottsater et al. did not find a decrease of neopterin levels after six months of
secondary prevention with the same drug [39]. There is evidence that supports a role for monocyte activation in patients with hypertension [40]. In healthy blood donors a small, but highly significant correlation was found between neopterin levels and diastolic blood pressure [33]. No data have been published on patients with hypertension.

A remarkable, but consistent, finding is that smokers have lower levels of neopterin [33;34]. An explanation for this apparently immunomodulating effect of cigarette smoking has not yet been given.

Hyperhomocysteinemia is an independent risk factor for cardiovascular disease. It is also associated with enhanced oxidative stress in patients with cardiovascular disease. Homocystein levels were related to neopterin levels in both patients with a disturbed glucose metabolism and control subjects [41]. Intervention with folates resulted in a decrease of homocystein levels but not neopterin levels in patients with hyperhomocysteinemia and vascular disease [42].

Obesity is an emerging cardiovascular risk factor, especially when it is part of the metabolic syndrome. Neopterin levels were related to both body mass index and glucose levels [43]. Given the fact that adipocytes are known to be potent producers of pro-inflammatory cytokines [44], this may not come as a surprise. Another feature of the metabolic syndrome, hyperinsulinemia, did not lead to elevation of neopterin levels in female subjects who underwent euglycemic hyperinsulinemic clamping [45].

**Neopterin in coronary artery disease**

Initially, neopterin emerged as a marker of an enhanced cell-mediated immune response in a variety of diseases such as HIV/AIDS and other infectious diseases, autoimmune diseases, transplant rejection and a number of malignancies [46]. Several authors have investigated the value of neopterin in patients with coronary artery disease.

Tatzber *et al.* [47] were the first to note that in hospitalised patients with peripheral vascular disease neopterin levels were higher than in non-hospitalised patients. They also found that patients with peripheral vascular disease had higher levels of neopterin than patients with coronary artery disease. In a later report, no difference between neopterin levels in patients with coronary artery disease was found [48]. Thirteen patients with severe coronary artery disease (≥75% stenosis at two or more sites as proven by coronary angiography) were compared to 11 males without or with minimal coronary atherosclerosis. Although the first group was older no differences in levels of neopterin (8.0 ± 2.7 versus 7.6 ± 2.5 mmol/L) as well as antibodies against oxidized LDL, lipid levels and fibrinogen levels were found. In a larger group of 21 patients with acute myocardial infarction (age 66 ± 1 5 years), 62 patients with chronic stable coronary artery disease (age 61 ± 8 years) and 40 healthy controls (age 35 ± 13 years) outspoken differences in levels of neopterin are found [49]. The highest levels are seen in patients with acute myocardial infarction but also patients with stable CAD have elevated levels. However, data were not corrected for age, and smoking behaviour is not mentioned. Similar results were reported by Gupta *et al.* [50] who found an elevated neopterin/creatinine ratio in 10 male patients who suffered an acute myocardial infarction and 17 patients with unstable angina pectoris as compared to 60 patients who had a previous myocardial infarction and 42 healthy controls. However, Auer *et al.* were not able to reproduce these results in a comparable study with 25 patients suffering from unstable angina or acute myocardial infarction, 30 patients with stable CAD and 60 healthy controls [51]. Thus, only limited information on neopterin in patients with different stages of coronary artery disease is present. The current reports include only a limited number of patients and the results are inconsistent.
Neopterin levels are thought to reflect the extent of coronary artery disease. Gurfinkel et al. [52] reported that, in patients with UAP, those who had elevated levels of neopterin were more likely to have a non Q-wave myocardial infarction as compared to those who had UAP but normal neopterin levels. Also, patients with elevated neopterin levels suffered more severe forms of angiographically determined atherosclerosis. Comparative results were reported by Garcia-Moll et al. who found a higher number of complex coronary artery lesions in patients with UAP who had elevated levels of neopterin [53], and by Erren et al. who related neopterin together with other markers of inflammation to the extent of coronary atherosclerosis [54].

Few have addressed the prospective value of neopterin levels in patients suffering from coronary artery disease. Women with stable angina pectoris and elevated levels of neopterin, had more cardiovascular adverse events during a follow-up period of one year. Patients with UAP had higher levels of neopterin at baseline as compared to women with stable disease, but, in these patients, neopterin levels did not prove predictive for an adverse outcome [55].

**CONCLUSIONS**

Atherosclerosis is an inflammatory disease. Neopterin, a marker of monocyte/macrophage activation is related to cardiovascular risk factors as well as the clinical presentation of cardiovascular disease. Moreover, neopterin is thought to reflect the extent of atherosclerosis in patients with coronary artery disease. Only one report established the prospective value of neopterin as a predictor of adverse events in women with stable angina pectoris. Since other markers of inflammation such as C-reactive protein, are currently advocated as clinical tools to determine cardiovascular risk in patients, it is necessary to establish a possible role of neopterin as a risk marker in these patients as well.


