Circulating cells: the biofactory for markers of atherosclerotic disease

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This editorial refers to ‘Interleukin-8 is increased in the membrane of circulating erythrocytes in patients with acute coronary syndrome’† by D.N. Tziakas et al., on page 2713

Atherosclerotic cardiovascular disease remains the number one killer of the ageing population in Western society. The natural history of the disease is unknown, and clinical events may strike without any warning. There have long been efforts to discover new biomarkers and to validate surrogate end-points to diagnose patients at risk for acute manifestations of atherosclerotic disease and to test treatment efficacy.

There is increasing evidence that suggests cross-talk between the diseased vascular tree and subsets of circulating cells. Functional, molecular, and structural characteristics of circulating cells may serve as biomarkers for initiation and progression of atherosclerotic disease. Several groups reported that the interleukin-6 (IL-6) release of circulating monocytes following in vitro lipopolysaccharide stimulation is strongly increased in patients suffering from unstable coronary syndromes compared with stable patients.† Animal studies demonstrated altered peritoneal macrophage function after myocardial infarction which was restored during endurance training. This demonstrates that these functional tests could also serve as a surrogate measure of disease progression or regression.‡ It has also been demonstrated that leucocyte Toll-like receptor responsiveness decreases acutely following vascular injury and increases chronically in relation to myocardial ischaemia.† Although these studies identify the circulating cell as a promising target in the field of biomarker discovery, the search for circulating cell-related biomarkers is still in its infancy due to the lack of cohort studies with systematic storage of fractions of circulating cells.

In the field of oncology, and specifically haematological malignant disorders, the circulating malignant cell has long been considered as a diagnostic biological entity for disease recognition. In immunology, T-cell subsets have been studied as markers for disease progression. In atherosclerotic disease, studies on circulating cell-related markers and functional tests are scarce, with the exception of haemostatic-related disorders. Leucocytes and thrombocytes have long been causally related with atherogenesis and vascular thrombotic occlusion. More recently, an increased appreciation has been noticed for the erythrocyte as a cell that is involved in atherosclerotic plaque destabilization. Arbustini et al.§ were the first to report that glycophorin A, a cell membrane marker for erythrocytes, was observed in atheromatous tissue in the arterial tree. They studied the composition of pulmonary artery plaques obtained from patients suffering from thromboembolic and plexogenic pulmonary hypertension. The atheroma-rich lesions were mainly observed in chronic thromboembolic pulmonary hypertension and stained strongly positive for the erythrocyte-specific membrane protein glycophorin. A few years later, the role of erythrocyte membranes in plaque destabilization was confirmed in a study in human coronary plaques.¶

About 40% of the weight of the erythrocyte is composed of lipid. The red cell membrane is comprised of a cholesterol-rich phospholipid bilayer. Cholesterol is intercalated between the phospholipid molecules. The ratio of cholesterol and phospholipid content is related to the fluid properties of the erythrocyte membrane. The significant staining for glycophorin A in lipid-rich lesions supports the concept that some of the free cholesterol present in the necrotic core of atherosclerotic plaques has its origin in the red cell membrane which is 1.5–2.0 times richer in cholesterol than any other cell.

The cholesterol content in the membrane of the erythrocyte may vary among individuals and was found to be elevated in patients who suffered from an acute coronary syndrome.¶ Moreover, increased erythrocyte membrane levels of the omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid have been inversely associated with risk for sudden cardiac death.¶

In their study, Tziakas et al. demonstrate that erythrocyte membrane IL-8 is elevated in patients with acute coronary syndromes compared with those with chronic stable angina.¶ They studied

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erythrocyte membrane IL-8 and plasma IL-8 levels in patients suffering from acute coronary syndrome, stable angina, and control patients. Erythrocyte-bound IL-8 revealed a significant increase in the patient group that had suffered from an acute coronary syndrome. This result gives rise to the idea that in addition to a role in plaque cholesterol deposition, the extravasated erythrocytes may propagate an inflammatory cascade within the atherosclerotic plaque. This result further supports the concept that the erythrocyte is more than an innocent bystander transporting oxygen.

With respect to the diagnostic value, the observations by Tziakas et al. merit careful consideration. First, blood samples were studied following an event, and the observational study design does not allow inferences regarding the diagnostic properties of IL-8 in the erythrocyte membrane. Secondly, blood samples were obtained at ~15 days after myocardial infarction. The authors considered the delay as an advantage since plasma IL-8 and erythrocyte-bound IL-8 levels are changing constantly for a few days after the acute event and that a 2-week delay would represent a steady-state condition. However, this argument can also be used against the suggestion that membrane-bound IL-8 is causally related to an event or could be used as a prognostic marker for disease progression. The delay and the strong fluctuations of IL-8 levels early after myocardial infarction also suggest that the erythrocyte-bound IL-8 is a consequence rather than a causal factor in the development of acute coronary syndrome. Indeed, a decade ago, de Winter et al. demonstrated that IL-8 is released in the plasma after an acute myocardial infarction and subsequently binds to red blood cells, resulting in only a transient rise of plasma IL-8 and a more prolonged increase of erythrocyte-bound IL-8. The latter results suggest that high levels of IL-8 in the erythrocyte membrane will become evident after the acute coronary syndrome. It could be hypothesized that a prolonged increase in erythrocyte-bound IL-8 following an acute coronary event accelerates an intraplaque inflammatory response and serves as a biomarker for future secondary events, but this should be established in a longitudinal study.

Although the diagnostic value of the presented results should be interpreted with caution, the results provide supportive evidence that the pool of circulating cells is an important and relatively unrecognized source of biomarkers for progression of atherosclerotic disease. An increasing need exists for the construction of prospective longitudinal biomarker studies that include the collection of circulating cells. This is not common practice in large cohort studies since cell sorting and storage requires laboratory facilities and is more costly. However, the first cohort studies have been initiated where cells are considered as a source for diagnostic markers. As an example, in The Netherlands, the government decided to stimulate research and innovation in the field of biomarker discovery including an €18 million programme that will focus on the biomarker properties of circulating cells in the progression of atherosclerotic disease (www.ctmm.nl).

In summary, there is growing evidence pointing to the circulating cell as a sensor for atherosclerotic disease initiation and progression. Whether this sensor function can be measured on a functional or expression level and whether it will reveal significant diagnostic power needs to be established in well-designed prospective studies.

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References