In search of the grail: the never-ending story of biomarkers for coronary risk prediction

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This editorial refers to “Association between differential leucocyte count and incident coronary heart disease: 1764 incident cases from seven prospective studies of 30 000 individuals”† by J.G. Wheeler et al. on page 1287

"I was brought up in an older tradition. I was told: "before ordering a test decide what you will do if it is (a) positive, or (b) negative, and if both answers are the same don’t do the test. At the other end of the scale is the most recent innovation whereby a package of results of a dozen tests arrive at the bedside almost before the signs and symptoms have been elicited" A. Cochrane. Effectiveness and efficiency, p. 43.

The leukocyte has been recognised as a principal effector cell in the acute inflammatory reaction since the end of the 19th century.¹ Circulating leukocytes, especially the polymorphonuclear cells, adhere to microvascular endothelium in response to cytokines and other inflammatory mediators, such as leukotrienes, or to platelet activating factors before they migrate across the endothelium initiating the atherosclerotic plaque formation.

Inflammation is present in all steps of atherogenesis and all developmental phases of vulnerable plaques. Patients with unstable angina have activated leukocytes and extensive monocyte and neutrophil infiltration is seen in fissured, thrombosed plaques. In vitro and in vivo as well as recent epidemiological studies suggest numerous mechanisms through which leukocytes may affect the stability of the plaque in acute coronary syndromes. One such mechanism has been described recently whereby the leukocyte enzyme myeloperoxidase predicted major adverse cardiac events in patients presenting with acute chest pain to emergency departments.² The white cell count has also been shown to be a prognostic factor of further events in patients with already established coronary disease. Monocytes and leukocytes transfer the tissue factor activating factor VII and the coagulation cascade. In the presence of an injured endothelium, platelets will then form the thrombus.

In this issue of the Journal, Wheeler et al.³ report that total white cell, as well as granulocyte and neutrophil blood counts are associated with incident coronary heart disease, although circulating lymphocytes and monocytes did not show any significant association with coronary events. The authors explore this issue in a useful and comprehensive double fashion by presenting new longitudinal data from the US National Health Follow-up Survey and through a meta-analysis adding another six previously published cohorts which included differential leukocyte counts. What is the meaning of these results? Total peripheral white blood cell count has been known for quite a long time to be a predictor of coronary events⁴ and all-cause mortality. Therefore, the results of this new study should come as no surprise. There is less evidence for an association with neutrophils, even though Metchnikoff⁵ postulated long ago that neutrophils mediate tissue injury.

There are several limitations of Wheeler’s study³ First, coronary events were not validated with clinical or epidemiological criteria and the routine data source used for case ascertainment is uncertain. Second, there were almost 1450 persons excluded from the analysis due to unknown leukocyte counts, leaving an open door for bias estimates. Third, the results of the total white cell count meta-analysis should be looked upon with caution because other studies that did not include specific leukocytes but which studied total white cell count were not incorporated in Wheeler’s meta-analysis and therefore the odd estimates might also be biased. Fourth, significant heterogeneity of results was found between the cohorts included in the meta-analysis. There are many potential factors able to explain such heterogeneity of the association effect of total leukocyte counts on CHD. An important one is the proportion of women included in...
each cohort. Furthermore, three out of the seven cohorts only included men. Other sources of heterogeneity might be the length of follow-up and the methods used for cell counting. It is likely that the co-efficients of variation of manual versus automated coulter cell counting are different. Adjustment for different confounding factors or differences in socio-economic status are also potential sources of heterogeneity. With respect to the latter, it is noteworthy that only one of the studies included in this meta-analysis was adjusted for socioeconomic-status, and it was this one that obtained the most significant results. Smoking and infection are both noxious agents whose exposure is heavily linked to socio-economic status. Chronic infection by diverse agents has been linked to CHD. Other than infection, the most common reason for inflammation and increased circulating leukocytes is cigarette smoking. Results for all cell types, except monocytes, showed a decrease of relative risks by 10–11% after adjusting for cigarette smoking, illustrating the power of cigarette smoke as a promoter of inflammation. Interestingly, animals treated with fish oil do not present leukocyte adhesion in response to cigarette smoking. Current evidence also suggests that quitting smoking may have an almost immediate effect in normalising white cell count. It is unclear however if all types of systemic inflammation and chronic infection throughout the life course invariably lead to atherosclerosis.

A weakness of the study is the lumping of results for both sexes. Given the much lower incidence of coronary heart disease in women, one would expect that the systematic study by sex of all aspects of inflammation in the atherogenic processes is warranted. Sex hormones modulate the response to inflammation, as oestrogen receptors are also present in white blood cells. Oestrogen is protective against platelet aggregation mainly through the modulation of NO production and it downregulates a number of proteins important in the development of cardiovascular disease such as cytokines, several adhesion molecules, angiotensin converting enzymes, and others. Furthermore, recent promising studies have suggested that oestrogen bioactivity on neutrophils differs by sex, as women’s neutrophils showed a much increased expression of the oestrogen receptors α and β after exposure to 17-β oestradiol, while men’s neutrophil oestrogen receptor β did not. Wheeler’s meta-analysis was indeed adjusted for sex, but it is unknown whether the association was present for women. This is relevant since in the NHEFS cohort, stratified analysis by sex showed no significant associations. Undoubtedly, statistical power for women was probably too small in the study (number of coronary events not stated) although very surprisingly the range of confidence intervals were similar in both sexes.

Among the array of new inflammation markers identified during the last decade or so, there is only long-term follow-up evidence for a few. Leukocytes, hsC-reactive protein, and fibrinogen are among them. Are they all alike? HsC-reactive protein or neutrophils which is the best marker to use? The neutrophil’s half-life is short and hence they are a good marker of acute inflammation. However, a problem with leukocyte and neutrophil count is its unspecificity. Their rise is often an indication of infection or other systemic inflammatory processes and does not necessarily mean the presence of atherosclerosis. Conversely, would any clinician rule out coronary disease in a person with a “normal” neutrophil count? Hence its use as a biomarker to screen or diagnose coronary disease would be limited by the possibility of a high number of false positives and negatives. On the other hand, the hsC-reactive protein half life is longer but more sensitive to liver dysfunction, including that produced by chronic alcohol intake. Recent evidence from Iceland also suggests that hsC-reactive protein might not be as strong a predictor of coronary heart disease as other earlier studies have found. The real question then should be: does determination of white cell count (or any other biomarker of inflammation) adds anything to predicting coronary events or identifying high-risk individuals over and above the identification and prediction provided by the simple question, ‘do you currently smoke cigarettes?’ or ‘have you ever been diagnosed of diabetes or hyperglycaemia or of high cholesterol?’ This subject is still awaiting a proper analysis and answer.

A recent joint AHA/CDC statement provides useful criteria for the clinical rational use of new inflammation biomarkers. It is therefore to be expected that there will be no immediate uncritical application of leukocyte counts from bench to bedside, a clinical behaviour otherwise too commonly observed whenever a publication of a new molecular or cellular finding appears in the literature. It nevertheless goes without saying that further good epidemiological research on the new inflammation biomarkers is more necessary than ever to both complement the understanding of their contribution to the pathophysiological mechanisms leading to the generation of cardiovascular events and to establish beyond doubt their clinical value and general applicability.

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References