Most acute coronary syndromes (ACS) are caused by thromboses occurring over ruptured vulnerable plaques (1). While the role of classical risk factors in atherogenesis is consolidated (2,3), their direct effect in acute plaque rupture and thrombosis is unproven: ACS can occur in individuals without risk factors (4), and most individuals with coronary atherosclerosis do not develop ACS (5). The peripheral biochemical phenotype of patients with ACS is progressively diverging from that of patients with chronic ischemic syndromes, and although the biology of ACS is highly subordinated to local factors, risk is better predicted by new sensitive, specific biomarkers of plaque instability (6) than by classical risk factors. These include acute phase reactants, cytokines, markers of platelet activation and oxidative stress, angiogenic growth factors, increased cell adhesiveness, and matrix metalloproteases (7), and they are markers of ACS and players in plaque instability (e.g., high-sensitivity C-reactive protein [hs-CRP] actively participates in endothelial dysfunction, atherosclerotic plaque formation, plaque maturation, plaque destabilization, and eventual rupture) (8). High values of CRP are associated with rapid disease progression (9) and higher number of vulnerable plaques (10).

In this issue of the Journal, Tziakas et al. (11) propose the total cholesterol erythrocyte membrane (CEM) as a marker of clinical instability: CEM was found to be significantly higher in patients with ACS than in patients with chronic stable angina (CSA) (11). If confirmed in larger case and control series, CEM could become an easy, low-cost indicator of instability in clinical settings. Data on the relation between CEM and circulating levels of cholesterol are conflicting: in Tziakas et al. (11) patient population, CEM levels were unrelated to circulating levels of cholesterol, and similar results have been reported in patients with combined hyperlipidemia and in normal controls (12). Prior human (13) and experimental studies (14) suggested that CEM is in equilibrium with the plasma cholesterol concentration. The relation between CEM and circulating levels of cholesterol needs to be further investigated using a unique standardized method in order to establish population-based normal reference ranges.

The hypothesis that CEM plays a role in clinical instability is biologically plausible: the rupture of a vulnerable plaque to cause ACS depends at least partly on the size of the pultaceous core (15). Free cholesterol constitutes about one-fourth of total plaque lipids within the core, and it is present in significantly higher amounts in vulnerable and ruptured plaques than in stable lesions (16). Sources of free cholesterol in the plaques are apoptotic/necrotic foam cells and lipoproteins whose cholesterol is, however, mostly esterified (17). The main candidates for accumulation of free cholesterol in atherosclerotic plaques are erythrocytes whose free cholesterol content exceeds that of all other cells (18). Erythrocyte membranes consist of 40% lipids (triglycerides, phospholipids, and cholesterol), 52% proteins, and 8% carbohydrates. Cholesterol is wedged between phospholipid tails in the lipid bilayer. It stabilizes the membrane and improves impermeability to small, water-soluble molecules (18,19).

Proteins provide membranes with their specific, cell-related functions. Among the 50 proteins identified to date in the erythrocyte membrane, glycophorin A (GPA) is an integral protein expressed only in erythrocyte membranes. Glycophorin A influences elasticity, plays an important role in preventing red cell aggregation in the circulation, and contributes to the glycocalyx (20). In 2002, we showed that anti-GPA-immunostained material is a major component of the pultaceous core of the large atherosclerotic plaques that form in the pulmonary arteries of patients diagnosed with chronic thromboembolic pulmonary hypertension, a human spontaneous model of atherosclerosis in individuals without risk factors (21). In 2003, Kolodgie et al. (22) reported that erythrocyte membranes are present in the necrotic core of advanced coronary atheromas and that degree of anti-GPA immunostaining and iron accumulation correlated with the size of the necrotic core. Plaque growth and pultaceous core formation and progression may be due to red cells entering the plaque, through constant leakage of...
erythrocytes from neovascularization; through larger intraplaque hemorrhages from ruptured microvessels; or through thrombi from healed ruptures within the plaques (23). The presence of GPA in the cores of the atherosclerotic plaques is the signature of the erythrocyte-derived origin of part of the plaque core. The challenging question is the link between the higher levels of CEM in patients with ACS versus patients with CSA found by Tziakas et al. (11) and the local plaque events that cause ACS. These authors did not study plaques but patients, and the study does not demonstrate a correlation between the core composition of the culprit plaques and CEM. They did show, however, that CEM levels were associated with angiographically complex coronary lesions (11) that predict rapid disease progression and adverse prognosis in patients with established coronary artery disease (24). They hypothesize a link between CEM levels and clinical instability, which may reflect atheromatous plaque instability. Similar correlations have been shown for other ACS markers such as CRP (25).

Does the Tziakas et al. (11) hypothesis link plaque core size and composition with CEM? Plaque core formation and growth likely progress slowly. Accordingly, it is difficult to consider CEM as a potential active player in acute plaque events causing ACS. Local plaque factors influencing rapid size changes could include both inflammatory and hemorrhagic processes. The amount of red cells incorporated into the plaque, their total lipid composition and CEM content, as well as failure by the core microenvironment and macrophage activity to clear necrotic red cells could influence plaque core size and composition. Nonlocal factors could also contribute. Membrane lipids are major active players in fluidity, aggregability, adherence, and deformability of erythrocytes: fluidity decreases as the cholesterol content increases (12). Erythrocyte aggregability increases in myoccardial infarction, angina, inflammation, diabetes, hypertension, and stroke; adherence to endothelial cells increases in inflammation diabetes and stroke; deformability decreases in infarction, angina, inflammation, diabetes, hypertension, and stroke (26). Cholesterol erythrocyte membrane is a dynamic component of the erythrocyte membrane lipids that is subject to modifications associated with a variety of physiologic, pathological, and pharmacologic conditions: it has been reported to increase with age (27), chronic alcohol assumption (28), obesity in women (29), pregnancy-induced hypertension (30), and after cyclosporine A administration (31). Cholesterol erythrocyte membrane decreases after exercise (32), in nephrotic syndrome (33), rheumatoid arthritis (34), and cerebral hemorrhage (35).

Although widely investigated either as total cholesterol content or phospholipid/cholesterol ratio, CEM did not find relevant clinical applications. A simple, standardized, reproducible analytical procedure would facilitate further studies aimed at validating CEM as a marker of ACS and investigating the effects of drugs on membrane cell components. The low CEM levels in patients taking statins, shown by Tziakas et al. (11), suggest that statins could influence red cell membrane homeostasis and composition: the pleomorphic effects of statin at the cell membrane level and that of other drugs on CEM represent promising new directions for research.

Reprint requests and correspondence: Dr. Eloisa Arbustini, Centre for Inherited Cardiovascular Diseases, Molecular Diagnostics, Cardiovascular and Transplant Pathology, IRCCS Policlinico San Matteo, Piazzale Golgi, 2, 27100 Pavia, Italy. E-mail: e.arbustini@smatteo pv.it.


