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Relation of carotid plaque with natural IgM antibodies in patients with systemic lupus erythematosus

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Abstract Noninvasive carotid measurements have proven value in the estimation of future cardiovascular (CV) outcomes in systemic lupus erythematosus (SLE). Natural IgM-antibodies to phosphorylcholine (PC) epitopes can enhance apoptotic-cell clearance and induce anti-inflammatory pathways. Herein, we show that subclinical CV disease, as detected by carotid ultrasound, in a cross-sectional SLE cohort was associated with lower levels of IgM anti-PC, as well as lower levels of the ratio of IgM anti-PC/total IgM, compared to patients without plaque ($p = 0.004$ and $p = 0.02$, respectively). The IgM anti-PC/total IgM association remained significant after adjusting for age, cholesterol and hypertension. Adiponectin and sE-selectin were significantly elevated in patients with plaque, and statistical models showed that combining adiponectin, sE-selectin and IgM anti-PC/total IgM was better for predicting plaque than either test alone.
These results support the hypothesis that IgM-natural autoantibodies may inhibit atherogenesis, and confirm the utility of IgM anti-PC levels as a biomarker for subclinical CV disease.

1. Introduction

Systemic lupus erythematosus (SLE) is associated with a strikingly increased risk for premature cardiovascular (CV) disease that is a major contributor to early mortality [1]. Indeed, women with SLE between 35 and 44 years of age have a 50-fold greater risk of a myocardial infarction [1] than age matched healthy controls, and lupus patients have an overall 17-fold increased risk of a fatal CV event [2] that cannot be fully explained by traditional Framingham risk factors [1]. Ultrasound measurements of carotid intimal thickness (IMT) have become an accepted non-invasive measure of subclinical atherosclerotic plaques and increased risk of cardiovascular events [3]. In women with SLE who were without a history of CV

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events, plaques by carotid IMT at baseline were shown to be significantly associated with incident CV clinical events during a mean follow up period of 7.9 years [4].

Surrogate markers related to endothelial cell injury and apoptosis may have utility for identifying a CV risk population. In a recent report, the presence of carotid plaque in SLE patients, as assessed by measurement of carotid IMT, correlated with higher levels of soluble E-selectin (sE-selectin) and adiponectin [5]. E-selectin is known to play a role in mediating adhesion between endothelial cells and leukocytes. Increased levels of soluble E-selectin (sE-selectin) may reflect endothelial activation that occurs in inflammatory diseases [6]. Higher sE-selectin levels are associated with increased risk of cardiovascular disease in both lupus and non-autoimmune patients [7,8]. In contrast, the adipose-derived factor, adiponectin, is generally considered to be anti-inflammatory and atheroprotective, yet elevated adiponectin levels are often found in SLE patients, although the mechanistic implications are unclear [9].

A focus of the present study is the use of natural IgM autoantibodies as biomarkers, as the homeostatic and immunomodulatory properties of naturally arising antibodies (NAbs) to oxidation-associated neo-determinants have recently been characterized [10–12]. IgM antibodies that recognize phosphorylcholine (PC) and malondialdehyde (MDA) neo-determinants on apoptotic cells (AC) are common components of the immune system, and in murine studies the induction of anti-PC antibodies blocked the progression of atherosclerosis in hyperlipidemic mice [13]. Furthermore, these IgM anti-PC antibodies can also increase the in vitro and in vivo phagocytic clearance of ACs, inhibit inflammatory signaling in innate immune cells [10–12], and suppress disease in models of autoimmunity [10]. Of clinical relevance, in recent cross-sectional studies it was demonstrated that SLE patients with history of a CV event had significantly lower levels of IgM anti-PC antibodies compared to patients who were event free [14,15]. Furthermore, higher IgM anti-PC levels were also found to correlate with lower overall lupus clinical disease activity [14].

The current study was initiated to address the hypothesis that decreased levels of IgM anti-PC would be predictive of subclinical atherosclerosis. This was approached by evaluation of sera from a cohort of SLE patients who underwent studies of carotid IMT. In addition, previously identified serologic biomarkers, sE-selectin and adiponectin, were fit into the risk model.

2. Materials and methods

2.1. Patient population

The recruited patients were previously described [5]. All patients fulfilled at least four of the revised ACR classification criteria for SLE [16], provided consent and were enrolled under a protocol approved by the Institutional Review Board of the New York University School of Medicine.

2.2. Clinical measurements

105 SLE patients underwent carotid ultrasound and the presence of carotid plaque was assessed as previously described [5]. Briefly, following the recommendations of the American Society of Echocardiography Carotid Intima-Media Thickness Task Force for the identification of preclinical vascular changes, high resolution images of right and left common carotid arteries, internal carotid arteries and carotid bulbs were obtained by an experienced sonographer using a linear array transducer [17]. The presence of plaque was defined as $\geq$50% increase over background IMT in any arterial segment.

The clinical status of each SLE patient was assessed with the SELENA revision of the SLE disease activity index (SLEDAI) [18]. Complete blood profiles were performed by the NYU clinical laboratory.

2.3. Biomarker assays

Levels of sE-selectin and total adiponectin were measured by commercial assays, according to the manufacturer's instructions (R&D systems, Linco Research Inc, respectively). IgM anti-PC, IgM anti-MDA, IgG anti-PC, IgG anti-MDA, and total IgM were assessed by ELISA, as previously described [14].

2.4. Statistical analysis

The distributions of biomarkers and other quantitative patient and disease characteristics were compared between the two groups using descriptive summary statistics, boxplots and two-sided Mann–Whitney tests. If the variances of variables were skewed between groups, these values were first logarithmically transformed before analyses, as appropriate. Frequency distributions and Fisher's exact test were used to compare categorical variables. Non-parametric Spearman correlation coefficients were used to examine the pairwise associations between continuous variables. Multivariable logistic regression analyses were performed when adjusting for age, hypertension and cholesterol levels. Furthermore, to calculate odd ratios and identify the best model to predict plaques; IgM anti-PC, adiponectin, sE-selectin and IgM anti-PC/total IgM, were evaluated in logistic regression models using a stepwise selection method. Analyses were performed with SAS 9.3 software (SAS Institute Inc.) and a p-value < 0.05 in two-sided analysis was considered significant.

3. Results

Of the evaluated 105 SLE patients, 44 met the criteria for the presence of carotid plaque. With regard to the Framingham traditional risk factors, patients with plaque were older (49 ± 13 years compared to 37 ± 11 years, $p < 0.0001$), had higher total cholesterol levels (186 ± 41 mg/dl compared to 169 ± 31 mg/dl, $p = 0.02$), and more often had hypertension (56% compared to 23%, $p = 0.0009$) (Table I). In addition, SLE patients with plaque also had elevated levels of sE-selectin (80 ± 62 ng/ml compared to 54 ± 27 ng/ml, $p = 0.005$) and adiponectin (18.7 ± 9.0 μg/ml compared to 14.6 ± 9.4 μg/ml, $p = 0.01$) as previously reported [5]. SLE patients with plaque had significantly lower levels of the protective natural IgM anti-PC antibody compared to those without plaque (22 ± 27 RU/ml compared to 39 ± 47 RU/ml, $p = 0.004$). These results were not simply the reflection of total IgM levels since