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Serum Biomarkers of Inflammation, Fibrosis, and Cardiac Function in Facilitating Diagnosis, Prognosis, and Treatment of Anti-SSA/Ro–Associated Cardiac Neonatal Lupus

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ABSTRACT

BACKGROUND Cardiac manifestations of neonatal lupus (cardiac NL) include congenital heart block and cardiomyopathy. Several candidate biomarkers were evaluated in cases at risk for cardiac NL on the basis of potential roles in inflammation, fibrosis, and cardiac dysfunction: C-reactive protein (CRP); NT-pro-B-type natriuretic peptide (NT-proBNP); troponin I; matrix metalloproteinase (MMP)-2; urokinase plasminogen activator (uPA); urokinase plasminogen activator receptor (uPAR); plasminogen; and vitamin D.

OBJECTIVES Identification of maternal and fetal biomarkers associated with development and morbidity of cardiac NL should provide clues to pathogenesis with translational implications for management.

METHODS Cord (139) and maternal (135) blood samples collected during pregnancies at risk for cardiac NL were available for study. Levels of cord and maternal CRP, cord NT-proBNP, and cord troponin I were evaluated using multiplex assays. Cord and maternal vitamin D were assessed by liquid chromatography-mass spectrometry. MMP-2, uPA, uPAR, and plasminogen were evaluated using ELISA.

RESULTS Cord CRP, NT-proBNP, MMP-2, uPA, uPAR, and plasminogen levels were higher in cardiac NL-affected fetuses than in unaffected cases, independent of maternal rheumatic disease, season at highest risk of cardiac NL development, and medications taken during pregnancy. These biomarkers were positively associated with a disease severity score derived from known risk factors for mortality in cardiac NL. Maternal CRP and cord troponin I levels did not differ between the groups. Cord and maternal vitamin D levels were not significantly associated with cardiac NL, but average maternal vitamin D level during pregnancy was positively associated with longer time to postnatal pacemaker placement.

CONCLUSIONS These data support the association of fetal reactive inflammatory and fibrotic components with development and morbidity of cardiac NL. Following CRP and NT-proBNP levels after birth can potentially monitor severity and progression of cardiac NL. MMP-2 and the uPA/uPAR/plasminogen cascade provide therapeutic targets to decrease fibrosis. Although decreased vitamin D did not confer increased risk, given the positive influence on postnatal outcomes, maternal levels should be optimized. (J Am Coll Cardiol 2015;66:930–9) © 2015 by the American College of Cardiology Foundation.

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Neonatal lupus (NL) is a form of passively acquired autoimmunity, typically presenting as cardiac and/or cutaneous disease. The former disease is clinically identified as congenital heart block and/or cardiomyopathy (1,2). Injury to the developing heart is postulated to occur secondary to a proinflammatory and profibrotic cascade initiated following transplacental passage of maternal autoantibodies to SSA/Ro and/or SSB/La ribonucleoproteins

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(2–4). Detection occurs most often between the gestational ages (GA) of 16 and 24 weeks of pregnancy (2). Cardiac NL is associated with a significant risk of mortality (17%; most occurring in the fetal or neonatal period) and morbidity (70% of survivors require pacemaker placement) (5,6). The case fatality rate is greatest in those with lower ventricular rates in utero and disease that extends beyond the atrioventricular (AV) node, such as endocardial fibroelastosis (EFE), dilated cardiomyopathy (DCM), and hydrops fetalis (5,6). The estimated risk of cardiac NL in anti-SSA/Ro-positive mothers with no prior affected pregnancies is approximately 2% and is 6- to 10-fold higher following a previously affected child (7–10). The low penetrance rate suggests that anti-SSA/Ro antibodies are necessary but insufficient for the development of disease and that fetal reactivity and in utero environment are likely contributory.

Several of the candidate biomarkers that have been associated with inflammation, fibrosis, and heart dysfunction may play a role in the development, progression, and severity of cardiac NL. C-reactive protein (CRP), a sensitive marker of inflammation, has been associated with coronary heart disease in adults and is elevated in fetal hypoxia and neonatal sepsis (11,12). N-terminal pro-B-type natriuretic peptide (NT-proBNP) is used in screening and diagnosis of congestive heart failure in adults and has been associated with fetal heart dysfunction in umbilical cord blood and amniotic fluid (13,14). Troponin I is a sensitive and specific marker for myocardial damage and necrosis (15). Elevated concentrations of matrix metalloproteinase (MMP)-2, a proinflammatory and profibrotic factor that activates transforming growth factor (TGF)-β, have been found in adult heart failure patients (16). MMP-2 is stimulated by urokinase plasminogen activator/urokinase plasminogen activator receptor (uPA/uPAR)-dependent plasminogen activation and plasmin generation, which have been associated with cardiac NL in a previous univariate analysis (17). Vitamin D has been shown to act as a negative regulator in TGF-β signaling and fibrosis and has been associated with cardiac inflammation, fibrosis, and dysfunction in rats (18,19). Recently, on the basis of indirect evidence and seasonal time of the vulnerable period during pregnancy, it was speculated that vitamin D levels might be decreased in mothers during pregnancies of cardiac NL children (10).

Identification of fetal and maternal biomarkers that associate with the development and morbidity of cardiac NL should provide clues to pathogenesis, with translational implications for management. Accordingly, this study used a U.S.-based cohort of anti-SSA/Ro-exposed cardiac NL-affected and -unaffected pregnancies to evaluate candidate biomarkers in umbilical cord blood of fetuses and in maternal blood from the time of pregnancy. It was previously reported that CRP, NT-proBNP, and troponin do not cross the placenta; thus, increases in cord blood biomarkers can be attributed to fetal production (20–22). The higher masses of MMP-2 (74 kDa), uPA (49 kDa), uPAR (37 kDa), and plasminogen (91 kDa) likely preclude placental transfer, and active transport of markers of collagen metabolism has not been reported in humans (23). Furthermore, MMP-2 and the plasminogen activators are expressed in placental tissue, and fetal and placental concentrations of uPA and uPAR are significantly higher than in maternal blood (24,25). Fetal vitamin D is entirely dependent on maternal stores and, because 25(OH) vitamin D diffuses across the placenta, cord and maternal blood levels correlate closely (26).

ABBREVIATIONS AND ACRONYMS

DCM = dilated cardiomyopathy

EFE = endocardial fibroelastosis

GA = gestational age

MMP = matrix metalloproteinase

NL = neonatal lupus

RRNL = Research Registry for Neonatal Lupus

SLE = systemic lupus erythematosus

SS = Sjögren's syndrome

uPA = urokinase plasminogen activator

uPAR = urokinase plasminogen activator receptor

METHODS

STUDY POPULATION. Subjects were identified from the Research Registry for Neonatal Lupus (RRNL), described elsewhere (27). The Institutional Review Board of the New York University School of Medicine approved the evaluation of deidentified information. Briefly, enrolled mothers have anti-SSA/Ro and/or

America LFA Lifeline Program. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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