EDITORIAL COMMENT

Cord Blood Samples

A Less Explored Tool in Early Diagnosis of Neonatal Cardiovascular Disease*

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At 7 to 8 weeks gestation, intervillous placental flow marks the early developmental processes of the human fetal-maternal interface. Connecting channels develop between maternal myometrial spiral arteries and lacunae in the wall of the implanted cytoblast. After connecting to the maternal circulation, exposure to allergens or antigens can occur and can stimulate an immune response in the developing fetus. The exact mechanism of how antigens cross from the maternal to fetal circulation is not fully understood. There is evidence that transplacental antigen transfer occurs as immune complexes via receptor-mediated transport across the syncytiotrophoblastic membrane and endothelium of vessels in fetal villi (1) (**Figure 1**). In order to protect from prenatal antigens, maternal immunoglobulin G (IgG) antibodies can, via placental transfer, reach the fetus to provide protection to the infant while his/her humoral response is inefficient. Interestingly, IgG seems to be the only antibody class that significantly crosses the human placenta, a process that is facilitated by the neonatal Fc receptor (FcRn) for IgG. Furthermore, fibroblasts and Hofbauer cells (i.e., placental macrophages) are found in the villous stroma and are presumably involved in the binding and trapping of immune complexes (1). In this regard, it is interesting to note that the anti-SSA/Ro or SSB/La antibodies belong mainly to the IgG and IgM class (2), a feature that may explain why specifically these antibodies may induce damage to the fetus, leading to neonatal lupus (NL).

Cardiac manifestations of NL include advanced condition defects and occasionally cardiomyopathy with a high morbidity and mortality rate. In this regard, a study by Ismirly et al. (3), which included 325 offspring exposed to maternal anti-SSA/Ro antibodies presenting with NL, showed a case fatality rate of 17%, with one-third of the cases dying in utero and a high post-natal mortality rate, too, because survival at 10 years for children born alive was >85%, with the majority of deaths occurring within the first year of birth.

In this issue of the *Journal*, the same team, this time led by Saxena et al. (4), report performing a carefully conducted, complex study presenting the first evidence of maternal passively transferred autoimmunity. Subjects were identified by the U.S. Research Registry for Neonatal Lupus, which enrolled mothers with anti-SSA/Ro and/or SSB/La antibodies and having at least 1 child with NL. Maternal and cord blood in cases with cardiac NL or unaffected pregnancies were compared. Plasma markers of inflammation, cardiac performance, and fibrosis were correlated using a rigorous score for fetal outcome. In total, cord blood was obtained from 139 anti-SSA/Ro-exposed fetuses, 42% (n = 59) with cardiac NL, and 54% (n = 75) without NL. Because the plasma markers for fetal reactive inflammatory and fibrotic components were elevated in anti-SSA/Ro-exposed fetuses, the authors conclude that they are possibly contributing to the development of cardiac neonatal lupus. A control group of fetuses not exposed to SSA/Ro and/or SSB/La antibodies was not included. This is regrettable because it would have
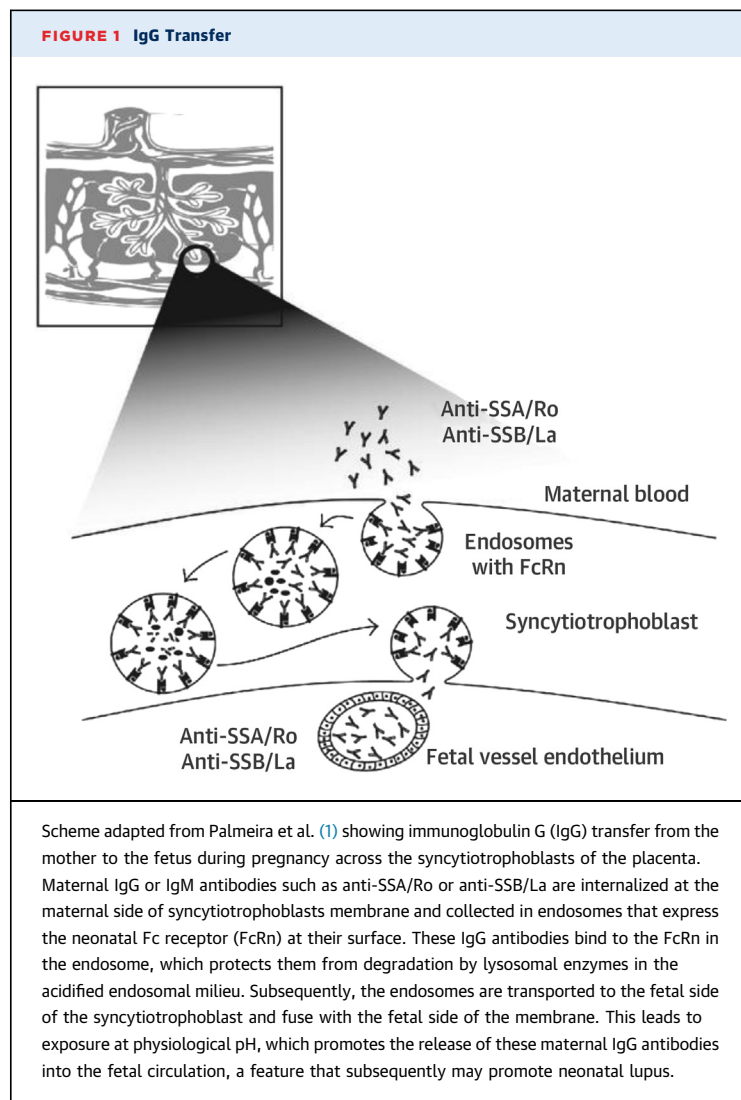
allowed a better interpretation of the individual values of the markers measured.

This is the largest repository reported from neonates exposed to maternal autoantibodies supporting the role of tissue inflammation in the pathogenesis and morbidity of cardiac NL. However, as the authors correctly speculate, other factors such as fetal reactivity and the in utero environment also are likely to contribute, because the estimated risk of cardiac NL is approximately 2%, and about 10% when following previously affected pregnancies in SSA/Ro antibody-positive women (3).

It is important to note that placental transfer of maternal IgG antibodies to the fetus provides protection to the infant whose own immune response is inefficient (2). Interestingly, SSA/Ro antibodies largely belong to the IgG antibody class, which is actively transmitted by the FcRn receptor system expressed on syncytiotrophoblast cells from the maternal circulation to the fetus (2), a feature that may explain why fetuses from SSA/Ro and/or SSB/La antibody-positive mothers have a higher risk of NL.

An observation in the present study is that troponin-I levels are not elevated in cord blood from NL neonates. Interestingly, anti-SSA/Ro and anti-SSB/La antibodies have been shown by Clancy et al. (5) to induce cardiomyocyte apoptosis. Because apoptosis is a controlled pathway of cell death, it may not lead to a substantial release of cardiomyocyte proteins. The study by Clancy et al. (5) also notes that the subsequent phagocytosis of apoptotic cardiomyocytes, that naturally would take place, may be blocked, leading to inflammation, scarring, and fibrosis, features that are typically observed in cardiac NL.

There is evidence from a few other studies that cord blood can be used for early detection of neonatal cardiovascular disease. Llurba et al. (6) reported on impairment in angiogenic biomarkers in maternal and cord blood associated with fetal congenital heart disease (CHD). Interestingly, in the plasma of women pregnant with a fetus with a major congenital heart disease, the antiangiogenic vascular endothelial growth factor receptor (VEGF) 1, also called soluble fms-like tyrosine kinase 1 (sFlt-1), was significantly higher. The interlinked, platelet-derived, proangiogenic factor placental growth factor (PIGF) was significantly lower. Fetuses with CHD also had higher cord blood levels of sFlt-1 and soluble engolin. Examination of the heart tissue from the fetuses with CHD showed a significant increase in markers of chronic hypoxia and antioxidant activity, as well as expression of VEGF and sFlt-1. The study also showed maternal serum PIGF being decreased and sFLt-1 increased at 18 to 37 weeks gestation, suggesting

FIGURE 1 IgG Transfer

Anti-SSA/Ro
Anti-SSB/La

Maternal blood

Endosomes
with FcRn

Syncytiotrophoblast

Anti-SSA/Ro
Anti-SSB/La

Fetal vessel endothelium

Scheme adapted from Palmeira et al. (1) showing immunoglobulin G (IgG) transfer from the mother to the fetus during pregnancy across the syncytiotrophoblasts of the placenta. Maternal IgG or IgM antibodies such as anti-SSA/Ro or anti-SSB/La are internalized at the maternal side of syncytiotrophoblasts membrane and collected in endosomes that express the neonatal Fc receptor (FcRn) at their surface. These IgG antibodies bind to the FcRn in the endosome, which protects them from degradation by lysosomal enzymes in the acidified endosomal milieu. Subsequently, the endosomes are transported to the fetal side of the syncytiotrophoblast and fuse with the fetal side of the membrane. This leads to exposure at physiological pH, which promotes the release of these maternal IgG antibodies into the fetal circulation, a feature that subsequently may promote neonatal lupus.

impairment in placental angiogenesis. The authors, therefore, speculate that placental hypoxia due to abnormal angiogenesis may cause fetal hypoxia, leading to abnormal heart development and low birth weight. Based on those and other observations, the term *cardio-placental syndrome* has been postulated (7). A study by Loukovaara et al. (8), demonstrated that in serum taken from cord blood at birth in pregnancies complicated by type 1 diabetes and hypertensive disorders, fetal hypoxia (measured by, for instance, amniotic erythropoetin and umbilical gas variables) was associated with elevated cord serum C-reactive protein levels. Hypoxia itself causes overproduction of sFlt-1 that could activate a cycle wherein high sFlt-1 levels inhibits angiogenesis and exacerbates placental hypoxia (7).

In conclusion, so far, only a few studies have analyzed the interrelation of maternal and cord-blood

serum biomarkers of inflammation, angiogenesis, and fibrosis, neonatal birth weight, and fetal and post-natal disease such as NL. Among them, this new study by Saxena et al. (4) provides significant evidence for a relationship between fetal and maternal biomarkers in women with maternal systemic lupus erythematosus. In addition, their study emphasizes a high predictive value of cord-blood serum for NL, a feature that should be explored in future studies, not only for NL, but also for the presence of other (cardiovascular) disease types, thereby allowing early detection and better management of fetal and post-natal disease.

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