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Respiratory Syncytial Virus Seropositivity at Birth is Associated with Adverse Neonatal Respiratory Outcomes

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Abstract

Background: More than 60 years since the discovery of the Respiratory Syncytial Virus (RSV), the effects of prenatal exposure to this virus remain largely unknown. In this investigation, we sought to find evidence of RSV seroconversion in cord blood and explore its clinical implications for the newborn.

Methods: Offspring from 22 pregnant women with a history of viral respiratory infection during the third trimester of pregnancy (RVI group) and 40 controls were enrolled in this study between September 1, 2016 and March 31, 2019. Cord blood sera were tested for anti-RSV antibodies by indirect fluorescent antibody assay. RSV seropositivity was defined as the presence of anti-RSV IgM or IgA, in addition to IgG in cord blood serum at 1:20 dilution.

Results: Anti-RSV IgG was present in all cord blood serum samples from infants born to RVI mothers (95% CI=82–100%), with 16 samples also having elevated titers for either anti-RSV IgA or IgM (73%; 95% CI=52–87%). No controls had evidence of anti-RSV antibodies. Eight (50%) seropositive newborns developed at least one respiratory tract finding, including RDS (N=8), respiratory failure (N=3), and pneumonia (N=1). RSV seropositive newborns also required more

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days on oxygen, had leukocytosis and elevated C-reactive protein ($p=0.025$, $p=0.047$ and $p<0.001$, respectively).

Conclusions: This study provides evidence of acute seropositivity against RSV in cord blood of newborns delivered from mothers with a history of upper respiratory tract illness in the third trimester. Cord blood seropositivity for anti-RSV IgA or IgM was associated with adverse clinical and laboratory outcomes in newborns.

Summary of the Article's Main Point: Previous studies have explored how maternal RSV infection leads to vertical transmission to the fetus. This study demonstrates serologic evidence of RSV infection in the cord blood of offspring from mothers with respiratory illness during late pregnancy, and characterizes the clinical outcomes associated with RSV seropositivity at birth.

INTRODUCTION

Even with repeated exposure to Respiratory Syncytial Virus (RSV) infection, immunologic protection is usually short-lived and incomplete, allowing RSV to re-infect the host throughout life ¹. In addition, studies of primary RSV infections show the neutralizing antibody response rapidly declines to pre-infection levels within months ². In this context, the role of viral respiratory illness during the physiologic immunosuppressed state that characterizes pregnancy is not well-defined, except for influenza ³. Several studies have shown that RSV disease may occur in mothers at any trimester of pregnancy ^{4–6}, but the consequences of this infection for the offspring remain unclear. Yet, mothers with respiratory illness are more likely to have poor maternal and perinatal outcomes than those without respiratory illness [7].

There is growing evidence pointing to extra-pulmonary involvement during RSV infection, including the detection of both viral antigens and genome in peripheral blood mononuclear cells of infected individuals ^{7–10}. As a result, recent studies have explored whether RSV can cross the placenta leading to vertical transmission of the infection from the mother's respiratory tract to the fetus ¹¹. First, Piedimonte et al. used recombinant RSV to infect pregnant rats and detected RSV genome and transgene expression in pulmonary tissues of 40% of their offspring ¹². The virus then persisted into adulthood in 25% of prenatally exposed rats. Importantly, the intrauterine RSV infection influenced expression and function of key neurotrophic pathways and affected the development of cholinergic nerves in the airways and lung tissues, leading to persistent bronchial hyperreactivity ¹². Subsequently, the same group reported the first documented case of congenital infection caused by vertical transmission of RSV from a mother with history of upper respiratory infection to her son born with acute bronchiolitis ¹³, while Fonceca et al. reported the presence of RSV genome in cord blood mononuclear cells and identified these cells as a potential reservoir for RSV ¹⁴.

Nevertheless, the ability of RSV to cross the human placenta and its impact on the respiratory outcomes of newborns needs further clarification. In this study we sought to determine serologic evidence of anti-RSV immunity in fetal cord blood of offspring with a maternal history of respiratory illness occurring during the third trimester of pregnancy, and also characterized the postnatal clinical outcomes associated with RSV seropositivity.

MATERIALS AND METHODS

Study subjects –

Between September 2016 and April 2017, women presenting for delivery to the Departments of Obstetrics and Gynecology at either University of Messina or University of Catania medical centers in Italy were screened for a history of respiratory illness. Informed consent was obtained from mothers who reported 2 of the following symptoms associated with influenza-like illnesses during the third trimester of pregnancy: fever, cough, and sore throat (**RVI group**). Control were also women presenting for delivery to the same Obstetrics and Gynecology units between September 2018 through March 2019 without any history of illness during their pregnancy (**Control Group**). The Institutional Review Boards of the University of Messina, University of Catania, and Cleveland Clinic Foundation in Cleveland, Ohio approved the study protocol. Data were securely stored and managed using the REDCap electronic data capture tools hosted at the Cleveland Clinic. Patient privacy was protected in compliance with the United States Health Insurance Portability and Accountability Act (HIPAA) and European Union General Data Protection Regulation (GDPR).

Participants provided comprehensive medical history information through completion of a detailed demographic and medical questionnaire. Thereafter, all subjects underwent routine obstetric examination. Maternal serologic screening for Hepatitis C Virus (HCV), Hepatitis B Surface Antigen (HBsAg), Human Immunodeficiency Virus (HIV), and Group B Streptococcus (GBS) were obtained. Mothers were excluded if they had diagnosis of any other infection, history of severe immunosuppression (e.g., HIV infection, transplantation, or malignancy), or used immunosuppressive medications. Clinical data reported from the studied newborns were restricted to findings during the initial hospital stay following delivery.

Clinical definitions –

Demographic, laboratory, radiologic, and clinical data of participating newborns were collected during their entire hospitalization. Previously published definitions for prematurity, low birth weight, intrauterine growth restriction (IUGR), atopy, transient tachypnea of newborn (TTN), respiratory distress syndrome (RDS), and bronchopulmonary dysplasia (BPD) were used^{15–23}.

RSV serology –

After delivery, fetal cord blood was collected for RSV serology as described previously²⁴. Briefly, the last 10–15 cm of the umbilical cord was disinfected with iodine prior to removing the clamp and 5 ml of blood was collected by gravity less than 10 min after the cord was sectioned with disinfected scissors. Serum was prepared by centrifugation at 2,650 *g* for 20 min, and aliquots were stored at –80°C until use. Anti-RSV IgA, IgM, and IgG antibodies were quantified using an immunofluorescence assay (Euroimmun, Padova, Italy) following the manufacturer's instructions. Positivity for RSV antibodies was determined based on previously published criteria: <1/20 dilution was considered negative, 1/20 positive, and 1/140 strongly positive²⁵. Cord blood serum samples with positive RSV IgM

and/or IgA in addition to positive IgG were considered seropositive for this study. This definition of neonatal seropositivity is similar to those used for diagnosis of other congenital infections including rubella, toxoplasmosis and parvovirus^{26–28}.

Statistical analysis –

Data were expressed as medians and quartiles for continuous variables and counts and percentages for categorical variables. Study groups were compared based on clinical characteristics and outcomes using the Wilcoxon rank sum, Chi-square, or Fisher's Exact tests as appropriate. The Agresti-Coull method was used to estimate 95% confidence intervals for the prevalence of RSV antibodies. All tests were two-tailed and performed at a significance level of 0.05. The SAS 9.4 software (SAS Institute, Cary, NC) was used for all analyses.

RESULTS

Between September 1, 2016 and April 30, 2017, a total of 22 pregnant women were enrolled in the study with a history of respiratory illness occurring in the third trimester of pregnancy. Forty controls were enrolled from September 1, 2018 through March 31 2019 who had no evidence of respiratory tract illness during their pregnancy. The majority of enrolled infants (84%) were born after 36 weeks' gestation with 6 infants born between 31 and 35 weeks' gestation and 3 infants born after 29 weeks' gestation. No differences in clinical characteristics were observed between the RVI and Control groups (Table 1).

RSV immunity –

Newborns born to mothers with a history of respiratory illness during pregnancy had serologic evidence of RSV immunity. All cord blood samples from the RVI group had titers for anti-RSV IgG 1:20 (95% CI = 82–100%), while 16 (73%; 95% CI = 52–87%) also had positive titers for either anti-RSV IgA or IgM, thereby meeting our criteria for RSV seropositivity (Table 1). Conversely, no cord blood from the Control group had any detectable anti-RSV antibody. The cord blood samples of 6 newborns (27%) were positive for both anti-RSV IgA and IgM. Samples positive for either IgA or IgM were more likely to have strongly positive IgG titers, with 14/16 (88%) having IgG titers 1/140, compared to 2/6 (33%) of those negative for IgA and IgM ($p=0.025$).

Clinical outcomes –

Infants born to RVI mothers had worse clinical and laboratory outcomes compared to controls. Eight (50%) RSV-seropositive newborns developed respiratory problems including neonatal respiratory distress syndrome (RDS; N=8), transient tachypnea of the newborn (TTN; N=5), apnea (N=5), respiratory failure (N=3), and pneumonia (N=1). In contrast, none of the RSV-seronegative newborns or the Control group were diagnosed with any respiratory pathology (Table 2). Also, 9 of the 16 (56%) RSV-seropositive newborns required supplemental oxygen, whereas none of the 6 RSV-seronegative newborns required oxygen ($p=0.025$). Furthermore, white blood cell (WBC) count and serum C-reactive protein (CRP) concentration were significantly higher in the RVI group compared to the Control group ($p<0.001$ for both) and between RSV-seropositive newborns compared to RSV-

seronegative within the RVI group ($p=0.047$ and $p<0.001$, respectively). None of the newborns enrolled in this study received surfactant or required invasive ventilation.

DISCUSSION

In this study, we show new evidence of RSV seropositivity in newborns born to mothers with influenza-like symptoms during the third trimester of pregnancy. While all infants born to RVI mothers showed positive titers for anti-RSV IgG, which likely represent transplacental transfer of maternal antibodies, 73% of cord blood samples collected at birth were also positive for either anti-RSV IgA or IgM. This serologic pattern is highly suggestive of intrauterine exposure to RSV, as these antibodies are not usually transferred from mother to fetus because of their larger macromolecular structure and typically do not appear until 3 to 7 days after infection.

Many respiratory viruses, including orthomyxoviruses²⁹, coronaviruses³⁰, and rhinoviruses³¹, can lead to transient viremia that is occasionally associated with severe or extrapulmonary disease. Likewise, RSV has been found in a variety of extrapulmonary human tissues both in immune competent and immune compromised subjects^{32; 33}. Therefore, it is conceivable that transient RSV viremia during pregnancy might lead to passage of live virions through the placenta with subsequent access to the fetus. Previous studies have provided proof of concept that vertical transmission of RSV infection is possible in an *in vivo* animal model, with detection of RSV genome, antigens, and transgene expression in the lung buds of fetuses born to rat dams infected with recombinant RSV at mid-gestation¹².

Maternal-to-fetal transfer of replicating RSV predisposes the offspring lungs to develop aberrant cholinergic innervation and smooth muscle contractility, leading to non-specific airway hyperreactivity. Furthermore, exposure of the pre-immune fetus to viral capsid proteins induces immune tolerance resulting in depressed Th1 and T-cell mediated anti-RSV immunity during early-life reinfection³⁴. Importantly, our group has recently documented that vertical transmission of RSV is possible in humans by reporting the case of a newborn admitted to the intensive care unit with respiratory distress. In this case, serology studies revealed that both mother and son were positive for anti-RSV IgG, IgA and IgM, while RSV RNA was amplified from the newborn's peripheral blood immediately after birth, confirming prenatal transmission of the infection¹³.

Given that RSV has a short incubation period, we focused on maternal disease occurring during the last trimester of pregnancy to assess the impact of RSV infection on the offspring when acquisition would be more clinically and serologically evident. Determining outcomes originating from maternal symptoms occurring in the first or second trimester would be difficult to discern, but findings in our rat model suggest that the implications for the fetus and offspring could be more severe due to the induction of immune tolerance by exposure to viral antigens during the pre-immune phase of ontogenesis³⁴. Indeed, other congenital infections occurring during fetal development are known to induce immune tolerance or altered immune response^{35; 36}.

Another novel and important finding of this study is that newborns with evidence of prenatal RSV exposure tend to have adverse pulmonary outcomes in the neonatal period. Indeed, we found that RSV seropositivity in the cord blood was associated with risk of pneumonia, RDS, and respiratory failure. Moreover, WBC count and serum CRP concentration were significantly higher in RSV-seropositive newborns compared to RSV-seronegative controls. Further longitudinal studies are needed to understand whether RSV exposure *in utero* leads to long-lasting consequences from direct injury or modified immune responses upon postnatal re-challenge.

There are some notable limitations to our study. In particular, we define as seropositive those newborns with high cord blood titers of anti-RSV IgA or IgM. The presence of IgA and IgM antibodies in cord blood has been used for determining presence of congenital infection to several pathogens^{26–28}. However, there is a notably high false positive rate associated with IgM, and to a lesser degree IgA, due to interference from high-titer IgG or cross reaction with proteins containing similar epitopes. In addition, cord blood seropositivity is not conclusive evidence of neonatal infection in infections like HIV, where IgA and IgM can be found in non-infected infants, especially soon after birth^{32; 37}. Nevertheless, in our study we also found that IgA and IgM positive cord blood samples are significantly more likely to have higher IgG titers, and correlate with adverse neonatal respiratory outcomes and with higher WBC and CRP levels. While maternal IgG can cross the placenta, IgM does not cross the placenta and is typical of more recent exposures. Additionally, several studies have demonstrated that IgA detected in neonatal blood is primarily of fetal origin³⁸. In normal development, fetal IgA is either undetectable or rises very slowly during gestation and fetal levels at term remain approximately 1,000 times lower than concentrations in the maternal circulation³⁹. Therefore, positive IgA together with positive IgM at birth suggests that the fetus might have been vertically exposed. Convalescent serology at 4–6 weeks, detection of RSV genome or antigens in cord blood, as well as pairing of maternal to cord blood serology would aid in confirming *in utero* RSV exposure. Lastly, serology against other respiratory viral pathogens were not tested in this study. Future serologic analysis against other viral etiologies will aid our understanding of respiratory outcomes in infants following later term exposure to respiratory viruses.

In conclusion, this study provides new evidence of acute seropositivity against RSV in the cord blood of newborns born to mothers with a history of respiratory illness during late gestation. RSV seropositivity at birth is associated with adverse clinical and laboratory outcomes in the neonatal period. More studies are needed to further define the direct and indirect effects of RSV infection occurring during intrauterine life, and its association with long-term respiratory sequelae.

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TABLE 1.

Newborns' characteristics and Cord Blood Outcomes.

Factor	Control (N=40)	RVI (N=22)	p-value
Gestational age (weeks), Median [Q1, Q3]	38[37,39]	38[36,39]	0.83 ^b
Caesarean Section, No. (%)	20(50)	17(77)	0.04 ^c
Birth weight (kg), Median [Q1, Q3]	3.05[2.63,3.38]	2.95[2.40,3.35]	0.63 ^b
Male gender, No. (%)	18(45)	14(64)	0.16 ^c
C-reactive protein, Median [Q1, Q3]	0.02[0.01,0.03]	3.85[2.70,4.80]	<0.001 ^b
White blood cell count (x1000), Median [Q1, Q3]	10.24[9.63,11.22]	15.20[14.20,16.40]	<0.001 ^b
RSV IgG, No. (%)	0	22(100)	<0.001 ^c
RSV IgM, No. (%)	0	10(45)	<0.001 ^c
RSV IgA [*] , No. (%)	0	12(55)	<0.001 ^c
RSV IgA or IgM [*] , No. (%)	0	16(73)	<0.001 ^c
RSV IgA and IgM [*] , No. (%)	0	6(27)	0.001 ^d
RSV Seropositive (Positive IgA or IgM plus positive IgG) [*] , No. (%)	0	16(73)	<0.001 ^c

^{*} Data not available for all subjects. Missing values: RSV IgA = 1, RSV IgA or IgM = 1, RSV IgA and IgM = 1, Active RSV infection (Positive IgA or IgM) = 1.

p-values:

b=Kruskal-Wallis test

c=Pearson's chi-square test

d=Fisher's Exact test.

TABLE 2.

Newborns' characteristics and outcomes by RSV seropositivity.

Factor	Newborns: RVI vs Controls			RVI group: RSV seropositivity		
	Control (N=40)	RVI (N=22)	p-value	No (N=6)	Yes (N=16)	p-value
Length of stay (days),Median [Q1, Q3]	4 [3,5]	3.5 [3,15]	0.20 ^b	3 [3,3]	5.5 [3,16]	0.12 ^b
Oxygen, No. (%)	1(3)	9(41)	<0.001 ^c	0(0)	9(56)	0.046 ^d
Pneumonia, No. (%)	0	1(5)	0.35 ^d	0	1(6)	0.99 ^d
Respiratory distress syndrome, No. (%)	0	8(36)	<0.001 ^c	0	8(50)	0.051 ^d
Respiratory failure, No. (%)	0	3(14)	0.041 ^d	0	3(19)	0.53 ^d
Transient tachypnea of newborn, No. (%)	0	5(23)	0.004 ^d	0	5(31)	0.27 ^d
Apnea, No. (%)	0	5(23)	0.004 ^d	0	5(31)	0.27 ^d
Doses of surfactant, No. (%)	0	0		0	0	
Total invasive ventilator days, No. (%)	0	0		0	0	
C-reactive protein, Median [Q1, Q3]	0.02 [0.01,0.03]	3.85 [2.70,4.80]	<0.001 ^b	1.95 [1.50,2.20]	4.35 [3.60,5.05]	<0.001 ^b
White blood cell count (x1000), Median [Q1, Q3]	10.24 [9.63,11.22]	15.20 [14.20,16.40]	<0.001 ^b	13.83 [13.10,15.46]	15.54 [14.51,16.92]	0.047 ^b

p-values:

b=Kruskal-Wallis test

c=Pearson's chi-square test

d=Fisher's Exact test.